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ENZYMATIC CONVERSION OF CELLULOSIC MATERIALS TO SUGARS AND ALCOHOL: THE TECHNOLOGY AND ITS IMPLICATIONS*

prepared by

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Summary

This techno-economic study deals with the production of sugars and alcohols from cellulosic materials. It covers such key subjects as: potential raw materials; the state-of-the-art on production technologies; the economics of extant processes; and infers implications for developing countries from the foregoing.

It is clear that a large number of cellulose-, starch- and sugar-containing plants can be processed to produce sugars and alcohols. Sugar-containing plants such as sugarcane, sweet sorghum and nipa palm are the best candidates for the high-yield production of alcohol fuel. Likewise, the starch-containing crops such as cassava, sweet potatoes, yams, taro and tannia are good candidates but require an additional step to break down starch to sugar. However, the emphasis of this report is on the major part of biomass containing cellulose and which, therefore, needs special treatment before it can be used to produce glucose and alcohols. To utilize cellulosic containing raw materials the following steps are necessary:

(1) Growth, harvest and delivery of raw materials to processing plants; or, alternatively, the collection and delivery of cellulosic "waste" products.

(2) Pre-treatment or conversion of the raw material by mechanical, physical, chemical or enzymatic methods to break down the cellulose to sugars and to modify or remove unwanted side-products, usually lignin and hemicellulose.

(3) Recovery and purification of sugars from reaction mixture.

(4) Fermentation of sugars to alcohol and purification by distillation.

(5) Treatment of process residues to reduce pollution and to recover potentially valuable side-products.

From the considerable R+D work carried out in all areas of pre-treatment it appears acid and enzymatic hydrolysis processes hold the most promise for developing countries. Though acid hydrolysis technology is more advanced, greater ultimate potential is seen in the enzymatic hydrolysis, which is, therefore, recommended for developing countries.

The economies of producing alcohol from cellulosic materials is at this time not favourable. Nevertheless, it is recommended that further R+D be undertaken in this area in order to accomplish one or more commercially attractive processes for producing alcohol from fermenting cellulose. If this was accomplished, the developing countries could gain in the following manner:

- Increase self-sufficiency since alcohols can to a great part substitute for petroleum fuels.
- Achieve a better balance of trade.
- Be able to increase employment, especially in rural areas.
- Achieve a higher level of technical competence in biotechnology and related areas.
- Be in a position to expand chemical industries.
- Establish an improved agricultural base by being able to utilize alternative crops and by being able to use what was previously termed agricultural wastes.

INTRODUCTION

1. It all began forty years ago during the Second World War. Hordes of microscopic fungi closed in on the American units stationed in the South Pacific. All cotton gear: tents, uniforms, knapsacks, cartridge belts were ruined after a brief period in the humid tropical climate. Too much cargo space was wasted just replenishing items which had become non-functional.

2. The situation was getting out of hand, so the officers called for help. As a result, several laboratories within the Armed Services were set up to investigate the nature of rotting, to find the causes, identify the invisible culprit, the way it operates, and to see what could be done to halt the damage.

3. The laboratories carried out a study which included investigating more than 10,000 microorganisms. This enormous task means growing each one on textile strips and testing tens of thousands of strips for any loss in tensile strength. It took several years to establish that the culprit was a special type of cellulose-destroying fungus. Therefore, it attacked cotton fabrics, since cellulose is the main component of cotton.

4. A few words about the chemical structure of cellulose. It is almost completely made up of sugar, or, more precisely, of glucose units. These are chemically bonded "head-to-tail" to form very long chains, containing hundreds and thousands of glucose units fixed into a rigidly ordered pattern rather like a crystalline latice. The result is a stable structure because the chains are cross-linked by so-called hydrogen bonds. In themselves the bonds are not very strong, but when blended with thousands of others they form a veritable monolith. As a result cellulose is not only insoluble in water, but its crystalline regions are virtually inaccessible to most chemical agents; neither water nor even strong acids can easily penetrate the "crystallites".

5. And now to the main point. The microorganisms we are examining attach themselves to cellulose-containing material and secrete a number of enzymes - biological catalysts.

6. Several thousand different enzymes exist in nature and each is a catalyst to a specific chemical reaction. In this case evolution has led to fungi able to produce enzymes that degrade cellulose. Termed "cellulases", they

act on cellulose in the immediate vicinity of the fungus, systematically cleaving the chemical bonds that link up glucose molecules into polymer chains, until glucose, the end product, remains.

7. Glucose, their main food, enables the fungi to multiply, grow and spread over new areas, secreting more enzymes until the available cellulose, or substrate, to use the biochemical term, is exhausted.

8. In nature this usually takes a very long time. Years elapse before a tree stump decays. In some cases the process can be greatly accelerated, as in the South Pacific story. However, the reaction could be speeded up even further. If the enzymes - cellulases - are isolated from the microorganisms, pooled and then added to the cellulose, the resulting glucose will not be consumed by the fungi, but will accumulate in the reaction mixture. There is another advantage; if cellulose-containing waste, not pure cellulose, were to be used as substrate, the problem of using the waste would also be solved. Glucose can be fermented to form ethanol and used as fuel instead of other chemicals, and additionally, the dehydration of ethanol produces ethylene - the basis of much of the modern chemical industry. Depending on its purity and the economic efficiency of the process, the glucose thus obtained could be widely used by the pharmaceutical, chemical, food and bioscience-based industries.

9. Cellulose heads the list of the world's renewable resources. The world-wide production of cellulose by nature runs into billions of tons annually. Mankind's use of these resources results in the production of very large quantities of cellulose-containing waste. If even a fraction of this waste was to be turned into useful products by enzymatic treatment, renewable supplies of edible carbohydrate and petrol substitutes would be substantially boosted. Hence, the assiduous research into these areas in recent years.

10. Generally speaking, cellulolytic enzymes are not the only agents capable of breaking down cellulose to sugars. It can also be done by subjecting cellulosics to a variety of physical or chemical methods. Physical methods include the steaming of cellulose or its γ-irradiation, though they are so far not technologically attractive. The best known chemical methods involve treating cellulose with strong mineral acids, such as sulphuric and hydro-chloric acids.

11. The use of acids in industrial application followed by the fermentation of the resulting glucose to alcohol has a longer than 70-year history. The first commercial application was in 1913 at Georgetown, South Carolina, where a plant was built to hydrolyze southern pine mill waste with 2% sulphuric acid at 175°C in rotary steam-heated digesters. The dilute sugar solution, produced at a 25 % yield, was fermented to yield 5,000 gallons of ethanol per day. A second plant was subsequently built in Fullerton, Louisiana. Both plants produced alcohol profitably until the middle 1920s, benefiting from extremely low cost sawdust waste and the lack of environmental controls. Eventually, a fall in the price of blackstrap molasses led to cheap supplies of sugar from this source and the two plants became unprofitable.

12. During the 1930s Scholler in Germany developed a process by which wood in a percolator was subjected to hydrolysis with successive batches of dilute acid. Wood was pretreated with 1% HCl and then digested with 0.5% sulphuric acid at 130 - 190°C for 18 - 24 hours in stationary digesters. By removing sugar as it formed yields amounted to roughly 50%, about twice the typical yield of earlier processes. Three such plants were built in Germany and used during the Second World War. Additionally, one was built in Korea, and one at Ems, Switzerland. In the Union of Soviet Socialist Republics (USSR), a percolation plant was built in 1935, with a further 40 being built after the Second World War.

13. The Bergius process, also development in Germany during the Second World War, used concentrated (42%) hydrochloric acid in special acid-resistant equipment. It gave cleaner syrup, but was expensive because the acid had to be recovered. Nevertheless, the sugars from the Scholler and Bergius processes were used to produce alcohol and to grow yeast for human consumption. By the end of the Second World War, approximately 9,000 tons of food yeast were being produced per year in Germany using these processes.

14. The Forest Products Laboratory of Madison, Wisconsin, in co-operation with the Cliff's Dow Chemical pilot plant at Marquette, Michigan, investigated acid hydrolysis of wood to sugar during and after the Second World War. The work resulted in a modification known as the Madison Wood Sugar Process which accelerated the hydrolysis process and produced higher yields. In this process, chopped wood waste was continously percolated with 0.4 - 0.6 sulphuric acid at temperatures

gradually rising to 150 - 185°C. The process gave 4 - 5% sugar syrup in 45 - 55% yield. After neutralization, the sugar syrup was concentrated to 50% molasses, which was fed to dairy cattle, used as a silage additive, and was also used to grow brewer's, baker's and food yeasts.

15. Immediately after the Second World War a plant incorporating the Forest Products Laboratory developments was built at Springfield, Oregon to produce ethyl alcohol for the manufacture of synthetic rubber via butadiene. After its completion, the Springfield plant demonstrated its functionality but in cost-effective terms it could not compete with petrochemical ethanol.

16. None of the above processes have succeeded as a commercial operation in the forty years since the Second World War. An exception is the percolation industry in the Soviet Union, where multifunctional, raw cellulosic materials have been used, resulting in the production not only of the alcohol but also of yeasts and furfurol (when lignin was used as fuel). Nowadays this industry consists of 40 full-scale plants with a maximum capacity of 1,000 tons of wood material per plant per day. The annual production from this technology is 400,000 tons of fodder yeasts and 195,000,000 liters of ethyl alcohol.

17. In recent years, the Brazilians have taken advantage of the Soviet experience for their wood hydrolysis plants. The Brazilian plants are not only making alcohol but also manufacture charcoal for use in the Brazilian steel industry. COALBRA, the Brazilian company responsible for building plants to make alcohol from wood, took its name from its two products, coke and alcohol. At the moment, COALBRA has a pilot plant under construction which will produce 30,000 liters per day of alcohol from wood. This work comes under the National Alcohol Programme (PROALCOOL), which was established in 1975 as a measure of partially substituting ethyl alcohol for petroleum derivatives. Impetus for PROALCOOL came from Brazil having to spend half of its export revenue - about US $10 billion - on buying petroleum from abroad.

18. In recent years investigators, mainly in the United States (US), have been working on the development of continuous hydrolyzers. These include Grethlein and Converse at Dartmouth, Church at American Can, and Rugg at New York University, all who have been developing continuous dilute acid hydrolysis

technology. The two-stage continuous hydrolysis has some important advantages over percolation, such as effective fractionation, improved by-product recovery, and higher sugar concentration. However, the prevalent thought today is that although such continuous saccharification may be ready for the first level of pilot scale investigations, it is not ready for commercialization.

19. It should be noted here that alcohol production through the acid hydrolysis of wood presents a series of disadvantages:

- to be economic, the scale of the process must be very large; to produce some 500,000 litres per day an area of nearly 600,000 hectares is required to maintain a constant supply of raw material and fuel;

- it is extremely capital intensive, requiring an investment per litre of industrial capacity which is double or triple those of other raw materials, while production costs are 50 to 100% greater;

- the process requires a large amount of sulphuric acid production (at the rate of one litre per three litres of alcohol produced);

- the process requires rather severe conditions, and, as a consequence, needs expensive corrosion-resistant equipment;

- severe conditions of the hydrolysis lead to a partial degradation of the formed glucose and, as a result, to a contamination of sugars by toxic by-products (eg. furfural) which in turn are poisonous both for microorganisms (fermenting yeasts, for example) and human beings.

20. The most promising alternative to the use of dilute acid is enzymatic hydrolysis, but it is certainly a mistake to think of it as a competing process. A future possibility is the development of a hybrid process which in turn uses dilute acid to accomplish a pretreatment-fractionation coupled with enzymatic hydrolysis to convert the residual (resistant) cellulose to glucose.

21. In other cases, a choice between acid or enzymatic hydrolysis of cellulosics depends on certain conditions, especially on the location of the respective plant, available raw materials and links with nearby chemical or biochemical factories able to supply mineral acids, culture broths etc.

22. Once the hemicellulose sugars and the lignin have been removed from raw materials such as wood, the remaining cellulose can be hydrolyzed. Both enzyme and acid catalysts are effective for this process. However, as a rule hydrolyzates after enzymatic conversion of cellulosics contain a substantially higher proportion of glucose over other products of cellulose degradation as compared to acid hydrolyzates. This may be particularly important when the aim is to produce pure glucose as food sugar, rather than ferment it to alcohol, since glucose can be enzymatically isomerized into fructose.

23. In the pages to follow, the following topics will be the background of the biotechnology of cellulose hydrolysis, the current state of scaling-up technologies in both developed and developing countries, available data on the economics of these processes on a pilot and semi-industrial scale, and potential for biotechnology of cellulose conversion into food and energy in the developing countries.

I. BIOTECHNOLOGY OF THE ENZYMATIC HYDROLYSIS OF CELLULOSE: A BACKGROUND

A. An Overview

24. The problems that must be resolved before a successful industrial process for glucose production from cellulose can be realized are the following:

(1) Selection of a readily available cellulosic raw material, the processing of which should be economically and technically feasible;

(2) Development of an effective pretreatment process which could significantly increase the subsequent rate of enzymatic hydrolysis and final product yield;

(3) Development of cellulolytic enzymes for glucose production at an optimum conversion;

(4) Optimization of the glucose production process from cellulosic materials;

(5) Development of an optimal enzyme reactor for the most efficient conversion of cellulose into glucose in terms of a continuity of operation, insoluble cellulose residues and enzyme recycling;

(6) Designing and operation of a pilot plant for the enzymatic production of glucose.

25. It has been established that in terms of action, cellulose-destroying enzymes can be subdivided into four groups regardless of which living organism they come from. These are one group of endoenzymes, two groups of exoenzymes and the fourth group consisting of cellobiases. "Endo" and "exo" are usually prefixed to the names of enzymes attacking polymer substrates in order to indicate the size of the part they are attacking. For instance, if an enzyme attacks the chemical bonds removed from the ends of a long polymer molecule it has the prefix "endo". It is "exo" when a short end group is cleaved off.

26. Hence, the longer the molecules making up the substrate (ie. the higher the degree of its polymerization), the less pronounced is the role of the exoenzyme in the initial stage of reaction; there are just not enough ends for it to attack. On the contrary, an endoenzyme is most active when the conversion of the polymer begins and weakens as the substrate molecules become shorter. That is why the degradation of polymer substrates in nature usually involves multi-enzyme complexes consisting of both "endo" and "exo" enzymes.

27. It can be seen that the endoglucanases begin the conversion process since the cellulose molecule consists of several thousand monomer units and the number of end glucose units in the native polymer is too small (compared to the number of intermediate glucosyl bonds in the cellulose) for the action of the exoenzymes to be noticeable in the initial period of the reaction. However, each successful attack of the endoglucanases breaks the polymer chain and forms two new ends in the shorter cellulose molecule, which can now be attacked by exoenzymes. In other words, the role of exoenzymes and the speed of their action increases as the endoenzymes degrade the cellulose.

28. The exoenzymes acting on the partially split cellulose are of two types. One cleaves off glucose, the end-product of the hydrolysis, while the other, because of the specific structure of its active site, cleaves off cellobiose which is a dimer (a coupled glucose molecule). The first exoenzyme is an exoglucohydrolase, the second, exocellobiohydrolase.

29. Finally, the cellobioses are split into two glucose molecules by the last of the enzymes in the cellulase complex - the cellobiases.

30. Before glucose can be obtained, the reaction has to pass through several stages, including the partial degradation of the native substrate. Reaction rate depends on the composition and quantity of the enzymes added to the cellulose, the state of the initial substrate (degree of its polymerization, degree of crystallinity, etc.), the quantity of native cellulose, the amount and the nature of constituents usually present in the native cellulose-containing material, the condition of the reaction, etc.

31. The formation of glucose and cellobiose during the course of the multienzymatic hydrolysis of cellulose takes place at a regular rate when pure amorphous or crystalline is the starting substrate. Curves reflecting the yield or conversion at any given moment of the starting material to glucose and cellobiose have, therefore, been worked out as well as the rate of accumulation of products. However, methods have not yet been developed to predict the dynamics of hydrolysis of heterogenous cellulosic material in an enzyme reactor.

32. There is another aspect of the matter. As noted above, in nature cellulose is mostly crystalline, and is rather resistant to all hydrolytic agents, including enzymes. To make crystalline cellulose amorphous and, therefore, more reactive toward cellulases, the very firm regular structure of its polymer chains must be broken. This can be done by subjecting the native materials to intensive milling, to treatment with phosphoric acid, or by dissolving it in special solutions. All these methods could be technologically feasible.

33. But before deciding on the method, it is worth determining how enzymatic hydrolysis is affected by the native material's crystalline structure. The problem can be defined more comprehensively: what is the impact of the main structural factors of cellulose (ie., specific surface, average particle size, degree of polymerization, and crystallinity) upon the rate of enzymatic hydrolysis.

34. This problem was recently resolved by a series of R+D projects in the U.S., the German Democratic Republic, Italy and the Soviet Union. Several dozen samples of cellulose, including native cellulose, were chosen for the experiments. All the pertinent structural parameters and the rate of enzymatic hydrolysis were registered and compared. Since the samples were of various origins, their structural factors also differed greatly. Thus, cotton was found to have the highest degree of polymerization - several thousands - while that of cotton ground in vibratory mill was lower - several

hundreds (depending on the duration of treatment and types of mills). Cellulose samples treated by cobalt-60 isotopes or on electron accelerators had the lowest degree of polymerization - up to 20. However, as it turned out, samples of identical degrees of polymerization displayed different rates of hydrolysis. In other words, other structural factors were more important than polymerization in determining the rate of the enzymatic reaction.

35. It was also found that the average size of the particles (all other factors being the same) of the initial cellulose was not crucial in enzymatic hydrolysis. Cellulose is a porous material with a developed, sponge-like internal surface. Thus, it is not the average size of the particles that determines its accessibility to enzymes, neither is it crucial to the efficiency of hydrolysis.

36. The accessible surface area is the main factor in determining the efficiency of the enzymatic attacks on insoluble cellulose. This has been demonstrated by the linear relationship between the rate of hydrolysis and specific surface (square metres of surface per gram of material). It is not, however, the only factor. The rate of enzymatic hydrolysis is inversely proportional to the degree of crystallinity (which can be readily determined by X-ray diffractometric analysis).

37. Incidentally, there is nothing unusual in a relationship between the degree of the crystallinity of cellulose and its specific surface area since both affect the hydrolysis rate to the same extent. The reason is that the greater the crystallinity the more packed the polymer chains of cellulose and the less accessible the surface of the substrate. Conversely, when the crystalline structure is destroyed, the inner chains of the cellulose give way and its accessible surface increases.

38. Consequently, this data may be used to predict the rate of enzymatic hydrolysis for virtually any cellulose sample, which must not, however, contain many heterogeneous admixtures (lignin etc.). It is simply a matter of measuring the specific surface area of the cellulose or the degree of its crystallinity. That, in turn, permits a standardization of cellulose-containing materials in industry; an important accomplishment. Thus, another bridge has been built between the structure of a substrate and its capacity to react - and the practical problem of technology, i.e., the enzymatic production of glucose from cellulose.

39. This paper emphasizes the problem of enzymatic conversion of cellulosics into sugars and of producing ethanol from the latter by microorganisms. The production of ethyl alcohol will be mainly considered in a later section dealing with scaling-up technologies for the enzymatic conversion of cellulose into fuels.

B. Raw Materials

40. Biomass in the form of agricultural and forest wastes accumulates every year in large quantities both in industrial and developing countries. This results in a deterioration of the environment and a loss of potentially valuable resources. Some of the potential biodegradable agricultural and agro-industrial cellulosic residues and their availability in several countries are listed below:

	By-products
Agricultural crops	Cotton stalks, rice straw, corn cobs, bamboo dust, wheat straw, maize stalks, banana stem, tapioca stem, castor stem, planted forests
Agro-industrial wastes from cotton-processing industry	Cotton linters, cottonseed hulls, cotton gin waste
Sugar industry	Bagasse pulp and pith
Rice-milling industry	Rice husk, rice bran
Jute industry	Jute stalk pulp, jute mill waste
Sawmill industry	Sawdust, wood chips
Coconut industry	Coconut husk, shell and pith

Potential Availability of Some Agricultural and Agro-Industrial Residues in the Soviet Union

	Million Tons Per Annum	Assumed Cellulose Content (%)	Million Tons
Wood-processing industry	61	40	24.4
Saw dust	15		
Wood chips	28		
Others	18		
Wheat straw	10	30	0.3
Paper-making industry and rayon manufacture wastes	0.1 - 0.2	90 - 100	0.1 - 0.2
Rice straw	1.5	30	0.45
Rice husk	0.4	33	0.13
Corn cobs	2.0	28	0.6
Cotton-processing industry			
Cotton stalks	6 - 10	35	2.1 - 3.5
Cottonseed hulls	1.5	50	0.75
Chiganak	0.8	40	0.3
Cotton linters	0.6	90	0.5
Cotton dust	0.1	90	0.9
TOTAL	84 - 88.1		30.5 - 32.0

Potential Availability of Some Agricultural Residues in India

	Million Tons Per Annum
Rice husk	18.0
Rice bran	3.2
Rice straw	59.2
Bagasse	52.1
Jute sticks	2.5
Cotton stalks	12.0
Cotton linters	1.2
Wood wastes	5.5
Coconut shell/coir dust	0.6
TOTAL	154.0

Source: Ref. (4)

Annual Production of Solid Cellulosic Wastes in the USA

	Million Tons	Assumed Cellulose Content (%)	Million Tons
Agricultural and food wastes	400	60	240
Manure	200	50	100
Urban refuse	150	45	68
Logging and other wood wastes	60	55	33
Industrial wastes	45	33	15
Municipal sewage solids	15	33	5
Miscellaneous organic waste	70	25	17
TOTAL	940		478

Source: Ref. (5)

Estimates of World Forest Resources - Growing Stock, 1978 and 2000

	Growing Stock Per Capita (m^3 biomass)	
	1978	2000
Industrial countries	142	114
Developing countries	57	21
Global	76	40

Source: The Global 2000 Report to the President, 1981, cit. Ref. (2), p. 153.

41. If just a fraction of these materials were to be converted into sugars and alcohol as well as into gas and feed protein, a significant contribution could be made to the overall problem of resource recycle and conservation. However, before using virtually any of these cellulosics it is necessary to convert it so it is more reactive toward cellulolytic enzymes. As indicated above (paragraphs 36 - 38), it is necessary to decrease its crystallinity and to increase specific surface and, of course, to remove as much as possible the non-cellulosic matrix of the cellulosic, which may otherwise sterically block the accessibility of cellulose to cellulase enzymes.

C. Pre-Treatment

42. Natural cellulose is a crystalline polymer generally associated in a matrix with hemicellulose and lignin and as such is highly resistant to enzymatic attack. Therefore, pretreatment is necessary. Most approaches separate the different types of pretreatment into mechanical, chemical, physical (other than mechanical), biological, and a combination of these methods:

Mechanical	Chemical	Physical
Ball milling	Phosphoric acid	Steaming
Two roll milling	Hydrochloric acid	Wetting
Hammer milling	Sulphuric acid	Pulping
Vibratory rod milling	Acetic acid	Freezing/thawing
Colloid milling	Ammonia	Radiation
Extrusion	Sodium hydroxide	
	Sulphur dioxide	
	Fe^{2+}/H_2O_2	
	Cadoxen	

Biological	Combinations
White rot fungi	Steam explosion
	High-temperature milling
	Alkali + ball milling
	SO_2 + steaming
	NO_2 + irradiation
	Biomechanical pulping

For all these methods it is assumed that the cellulosic material is already in a form which can be readily processed.

43. Most pre-treatment processes have energy requirements that depend on the severity of the process. Severe mechanical and/or thermochemical processes have substantial energy requirements and when combined with the energy requirements for product separation, can make some cellulosics bioconversion processes very inefficient. A special analysis performed recently by Datta from Exxon Research and Engineering Company, Linden, New Jersey, has shown that many of the proposed processes which use fine particles, severe conditions, etc., can consume a substantial amount of energy and lead to a generally inefficient process in terms of energy. Conversion processes that can use coarse particles, mild pretreatments, non-sterile conditions, etc., will have significant advantages in this respect.

1. Mechanical

44. Mechanical pretreatments (see paragraph 42) utilize sharing and impacting forces to yield a fine substrate possessing a low crystallinity index and high specific surface, thus enhancing its susceptibility to enzyme action.

Effect of Vibratory Milling on the Structure of Cotton Linters and on Their Conversion into Glucose

Duration of Milling (Minutes)	Size of Particles, (Microns)	Degree of Polymerization	Specific Surface, (m^2/g)	Degree of Crystallinity, %		Glucose Formation	
				Dry, Pre-parations	wet and Dried After Milling	Initial Velocity, (g/l/h)	Yield After 24 hrs (g/l)
0	Fibres	1100	0.17	85	86	0.09	1.8
2	32	870	0.27	65	69	0.16	2.0
2.5	22	420	0.27	-	-	0.25	3.5
5	19	510	0.28	-	-	0.41	5.2
7	19	490	0.32	-	-	0.44	6.0
10	19	430	0.30	30	54	0.61	6.6
15	18	320	0.45	-	-	0.57	6.5
20	17	280	0.54	25	52	0.56	6.2

Source: Ref. (6)

45. The use of fine substrate gives higher slurry concentrations, or higher bulk density, thus reducing the reactor volume. Using milling as a pretreatment method has the advantage of being relatively substrate insensitive but suffers from the major disadvantage of being energetically unfavourable (see below) and lignin remains to act as a significant inhibitor to enzyme accessibility.

Energy Requirement for Size Reduction of Municipal Solid Waste

Mesh Size	Particle Size (Microns)	Power Required (KwHr/ton)	Energy Used / Energy Content of Feed, %
40	420	100	6.3
80	180	330	21
100	150	400	25
200	74	1700	104
270	53	2900	180

Source: Ref. (7)

Energy Requirements for Size Reduction

Material	Size Reduction Process	Particle Size (Mesh)	Energy Required (KwHr/ton)	Energy Required / Energy Content of Feed, %
Hardwood chips	Cutting or shredding	10 - 30	20 - 40	1.3 - 2.5
Wood flour	Grinding	50 - 100	100 - 200	6 - 12
Fibre board (coarse pulp)	Disc mill		150 - 200	17 - 20
Newsprint pulp	Mechanical pulping		2200 - 2600	140 - 160

Source: Ref. (7)

46. The estimated cost of mechanical pretreatment of wheat straw may vary from US$ 0.01/kg for Fitz milling (model D comminutor, Fitzpatrick Company, Elmhurst, Illinois) to $ 2.24/kg for roller milling (based on the weight of the substrate).

Cost Analysis of Mechanical Pretreatment Methods and of γ - Irradiation Pretreatment

Type of Pretreatment	Yield of Sugar from Wheat Straw (g/kg)	Pretreatment Cost Based on Sugar ($/kg)
Ball milling, 8 h	260	5.82
Roller milling, 0.25 h	160	14.00
Fitz milling, fine	100	0.10
Extrusion, with pressure	64	0.16
γ-Irradiation	210	0.48

Source: Ref. (8)

47. Of the mechanical pre-treatments shown above, ball milling gives the most promising results in terms of hydrolysis rate and sugar yield. This pre-treatment is clean and easy to operate, but the long pre-treatment time makes its large-scale operation impractical.

48. The main difficulty with size reduction of cellulose is the fibrous nature of the material. This is evident from the comparatively low energy requirements for the size reduction of a brittle material such as coal (which takes only 5 - 7 KwHr/ton to reduce the particle size to 100 - 200 mesh by means of simple pulverization). For this reason, we find the energy content of feed as low as 0.2 - 0.3% compared with 25 - 100% for fibrous cellulose.

2. Chemical

49. Chemical pre-treatments have been used extensively as a means of lignin removal and to modify the structure of lignocellulosics. Conventional pulping processes, such as kraft, sulfite, and soda processes, are suitable for delignification, but these processes have been designed for removing lignin in order to preserve the quality of cellulose. However, these processes are too expensive to be used as bioconversion pre-treatments.

50. Another, more promising pulping process involves the use of SO_2, which causes a disruption in the lignin-carbohydrate association without the selective removal of either constituent. Typical process conditions are: temperature 120 - 150°C, 20 - 100 kg SO_2/ton of dry biomass and the final solids content of 25 - 30%. 600 - 900 kg of steam per ton of dry biomass is required when about 50% of the energy is recovered to heat the boiler feed water. This translates to 9 - 18% of the energy content of the biomass being processed. It was shown that pre-treatment by pressure cooking for 2 - 3 hours at 120°C in an SO_2 atmosphere resulted in nearly quantitative conversion of hardwoods to sugars while softwoods are only slightly less readily hydrolyzed. Apparently the presence of lignin in the pretreated wood substrate does not directly interfere with enzymatic hydrolysis since once the cellulose is loosened from the lignin matrix, enzymatic hydrolysis can occur even though the lignin is still present.

51. The most common chemical pretreatment is caustic swelling. Pretreatment with caustic soda leads to an increased surface area due to a swelling and disruption of the lignin structure. Generally swelling occurs in two forms. Swelling agents such as water act at the "intercrystalline" level of cellulose, with a volume change approximately equivalent to the volume of water, resulting in a minor crystalline modification of the substrate. "Intercrystalline" swelling agents penetrate the crystalline as well as the amorphous region of the cellulose component and lead to a new crystalline modification, which is more reactive toward cellulase enzymes.

52. Some limited swelling agents are sodium hydroxide, certain amines and anhydrous ammonia. Unlimited swelling, which leads to the complete solubility of the cellulose, is induced by concentrated sulphuric and hydrochloric acids, cupram, cuen and cadoxen. Although the unlimited swelling agents efficiently increase the accessibility of cellulose substrates there are still problems with product separation, chemical recovery and interference by lignin. It is doubtful that any of the unlimited swelling agents provide an alternative to a more economically feasible pretreatment such as dilute alkali swelling.

53. Acid pretreatment uses dilute acids such as hydrochloric (HCl), sulphuric (H_2SO_4), and phosphoric (H_3PO_4) to remove hemicellulose by hydrolysis without causing significant glucose formation. This has recently been discovered

by Grethlein of Dartmouth to be an effective pretreatment for enzymatic hydrolysis of substrates such as newsprint, corn stover, poplar and oak. Following pretreatment and subsequent enzymatic hydrolysis, glucose yields as high as 100% have been obtained. The pretreatment is carried out in a continuous flow reactor at temperatures around 200°C, acid concentrations less than 1 - 1.5% in weight, and reaction times to the order of 12 seconds. Increasing the temperature to 220°C achieves approximately the same glucose yields; however, a smaller proportion of the glucose is produced by the enzymatic hydrolysis because the higher temperature pretreatment converts 15 - 28% of the cellulose to glucose. Here it should be noted that one major shortcoming of acid hydrolysis processes is the low total yield of sugars (50 - 55%) owing to side product formation.

54. Another technique for chemical pretreatment is the oxidation of lignin by an oxidizing agent (for example, peracetic acid or Fe^{2+}/H_2O_2) which liberates cellulose for enzymatic hydrolysis. Considerable attention also has been paid to delignification using solvents such as ethanol, butanol, and acetone plus a suitable catalyst; the possibility of solvent recovery makes this alternative attractive. Although most chemical pretreatments are effective, waste chemicals are often difficult to dispose of or recycle.

55. The estimated costs of chemical pretreatment of wheat straw vary from US$ 0.4/kg for caustic pretreatment to $ 11.25/kg for ethylene glycol treatment. In comparison, the costs of mechanical pretreatments vary from US$ 0.01/kg to $ 2.24/kg (see paragraph 46).

Cost Analysis of Chemical Pretreatment Methods

Type of Pretreatment	Yield of Sugar from Wheat Straw (g/kg)	Pretreatment Cost Based on Sugar ($/kg)
Caustic - AC (autoclaved)	340	0.12
Peracetic acid	280	26.84
Sulfite - AC	250	1.26
Ethylene glycol - AC	240	46.53
Hypochlorite - AC	240	3.92
Sulphuric acid	140	0.78
Butanol	72	39.49
Control	70	

Source: Ref. (8)

56. Of the chemical pretreatments shown above, caustic pretreatment is a potential candidate for large-scale process development based on pretreatment cost, hydrolysis rate, and its sugar yield. Chemical pretreatments, however, have disadvantages which cannot be ignored. These include the required use of specialized corrosion-resistant equipment, the need for extensive washing, and the difficulty of disposing of chemical wastes.

3. Physical

57. Physical pretreatments of cellulosics involve primarily γ irradiation and high-pressure steaming, either with or without fast decompression. Actually, steaming with a sharp reduction in pressure couples physical and chemical pretreatments; it will be considered in the last section of this chapter (paragraphs 66 - 70).

58. Steaming of biomass in the 150 - 200^{o}C temperature range leads to increased enzymatic digestibility as a result of increasing pore size and the partial hydrolysis of hemicelluloses. The residence time at higher temperatures should be kept low to minimize reactions which produce by-products of a non-carbohydrate nature. According to some data and calculations, to reduce biomass containing 50% solids to a product containing 25 - 40% solids by steam treatment at 150 - 200^{o}C, approximately 450 - 1300 kg of steam would be required per ton of dry biomass treated. This method has not yet been proved effective in greatly increasing enzymatic hydrolysis.

59. γ - irradiation was not effective as a pre-treatment of relatively pure cellulose (see below), but it slightly accelerated (up to 1.5 - 3 fold) the enzymatic hydrolysis of lignocellulose substances such as wheat and barley straw and bagasse. Other mechanical or chemical pretreatments, on the other hand, accelerated the conversion of bagasse 7 - 10 fold.

Effect of γ -Irradiation on the Structure of Cotton Linters and on the Effectiveness of its Enzymatic Hydrolysis

Dose, Mrad	Degree of Polymerization	Degree of Crystallinity, %	Glucose Formation	
			Initial Velocity, g/l/hr	Yield After 48 hrs, g/l
0.0	1100	85	0.09	2.3
1.7	680	86	0.10	2.2
5.2	560	86	0.10	2.0
12.0	250	86	0.11	2.2
29.0	140	85	0.10	2.3
43.0	110	84	0.09	1.7
118.0	19	84	0.11	1.6

Source: Ref. (6)

4. Biological

60. Lignin-utilizing microorganisms have been used for biological pre-treatment of lignocellulosic materials. This idea was inherent in the original concept of "white rot"; first thought to involve only lignin degradation. However, as subsequently shown, soft and brown-rot fungi degrade cellulose and hemicellulose in preference to lignin while white-rot fungi destroy all three wood components at the same time, and remove the lignin faster than other polymers. Unfortunately, evidence indicates that white-rot fungi do not use lignin as a growth substrate, so a complete selective removal of lignin by these organisms seems impossible.

61. Nevertheless, partial delignification without cellulose loss is possible. Eriksson in Sweden has isolated cellulase-less mutants of white-rot fungi, which utilize a large fraction of xylan and mannan while degrading lignin from wood. For wood treatment with cellulase-less mutants of Phanerochaete sp. (= Sporotrichum pulverulentum) 35% of the xylan and all soluble substrates are utilized to degrade 30% of the lignin. At the same time the major problem of a 10 - 14 day phase still remains.

62. Because of the time factor, removal of a substantial amount of lignin by fungi has not been seriously considered. For example, lignolytic fungus Pleurotus ostreatus when used in a 10 - 20-day trial to delignify wheat straw partially and to increase its enzymatic saccharification yields, did not increase the saccharification yields of the residue over the control. The yield, however, did increase 4 - 5 fold after 50 days of fermentation. Clearly, further studies are needed to explore the potential of biological delignification. Meaningful economic analyses require pilot-scale studies, which have not yet been conducted.

5. Combined Pre-Treatment

63. Of the combinations of pretreatment methods briefly considered here, biomechanical pulping and steam explosion are particularly attractive.

64. The properties of wood after partial bio-delignification (see paragraphs 60 - 62) have been examined, paying particular attention to the energy requirements for subsequent mechanical pulping. Preliminary studies with mill refining show that removing even small amounts of lignin (2.1% of the original) from pine chips resulted in substantial energy savings (20%). Moreover, enzymatic digestibility of the material was improved because the strength properties of the resulting mechanical pulp were lower than those of untreated pulp at a given degree of refining.

65. Modifying biomechanical pulping involves partial delignification of coarse thermomechanical pulp (TMP) prior to "post refining" for final pulp production. When coarse TMP is treated with a white-rot fungus until it loses 2% in weight, a considerable decrease in energy consumption is observed in the post refining. Moreover, this decrease in energy consumption is accompanied by a loss in strength properties, especially when a wild-type rather than a cellulase-less mutant is employed. The fungal degradation rate of lignin appears to be faster in coarse TMP than in wood chips. Lignin degradation rates of an average 3% per day, over a 2-week period, have been observed; this, however, is preceded by a 3 - 4 day lag period for primary growth of the fungus in pulp. As with wood

chip treatment, technical problems with fungal treatment of TMP include maintaining correct environmental conditions on a large scale and the slow-paced degradation of lignin.

66. Steam explosion as a pre-treatment essentially involves steaming lignocellulosics at various temperatures, pressures and retention times with a sudden sharp reduction in pressure to expel the material from the vessel. This treatment opens up the fibre, renders the hemicellulose soluble in hot water and appears to some extent to depolymerize the lignin. When conditions are optimized, the lignin becomes readily soluble in dilute sodium hydroxide solution from which it can be recovered by acidification as an active chemical.

67. Steam explosion includes both physical and chemical pretreatments. The high-pressure steaming of moist lignocellulosic substrates results in a partial decomposition of some of the hemicellulose components into acids, mainly acetic acid, which, in turn, catalyze the depolymerization of hemicellulose and lignin. Su from General Electric found the highest conversion when aspen wood chips are steamed at 195°C for approximately 20 minutes in the presence of SO_2. Sulphur dioxide acts mainly as a catalyst.

68. Two Canadian companies also use steam explosion to pretreat lignocellulosics. Stake Technology operates a tubular, high-pressure, continuous reactor at temperature ranges of 200 - 240°C using various retention times. This equipment was initially developed for steaming hardwoods such as aspen and birch to make them digestible by ruminants. Other equipment, developed by the Iotech Corporation, uses a modified Masonite gun from which wood chips, after being steamed at approximately 200 - 250°C for 20 - 100 seconds, are exploded. The Iotech lignin from aspen wood becomes relatively soluble in ethanol after steam explosion and thus is a potentially valuable by-product for use as possible chemical feedstock or in wood adhesives.

69. The major advantages and disadvantages of using steam explosion as a method of pretreatment are as follows:

Advantages	Disadvantages
- Ability to separate wood into its three main components.	- Not yet suitable for softwoods.
- Relatively pure products at high yields.	- Steam-treated substrates first have to be washed to remove inhibitors.
- Acid hydrolysis gives 70 - 80% glucose yields of that theoretically possible.	- Produces a substrate of low bulk density.
- Enzymatic hydrolysis can give 100% conversion into glucose.	
- Lignin is produced suitable for conversion into chemical products.	
- Hemicellulose can be fully utilized and converted into liquid fuels.	
- Fermentation inhibitors are readily extractable.	
- Modular process may be employed with variable optimums for specific products.	

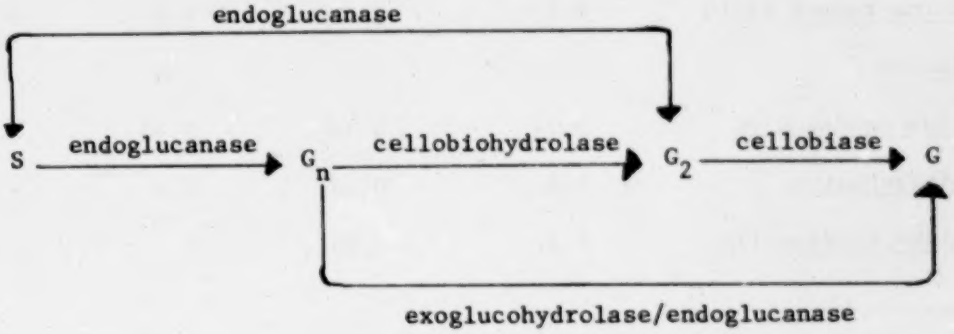
Source: Ref. (2), p. 219

70. Steam explosion has proven to be one of the most energy efficient methods of pretreating wood substrates as well as one of the most efficient methods for enhancing subsequent enzymatic hydrolysis of hardwood species.

II. ENZYMES

A. Composition

71. In the context of contemporary understanding, a cellulase complex contains four groups of enzymes (see paragraphs 25 - 30). The overall flow chart of the enzymatic hydrolysis of cellulose is as follows:



Here endoglucanase attacks native cellulose (S), which is amorphous or crystalline, and its action produces partially degraded cellulose (G_n). Then endoglucanase and/or cellobiohydrolase split the cellobiose units (G_2) off the ends of the insoluble celluoligosaccharides (G_n), and this cellobiose is later converted into glucose (G) by cellobiase. The final enzyme in the cellulase complexes splits off glucose directly from the ends of long oligosaccharides. This enzyme evinces so-called exoglucohydrolase activity and is in some cases an individual enzyme but in some complexes its fuction is performed by endoglucanases. In any case, this way of forming glucose, without including intermediate cellobiose hydrolysis, often plays a decisive role in the hydrolysis of cellulosics.

72. The composition of cellulase complexes in terms of the relative content of the individual cellulolytic components varies greatly from one biological source to another. Thus, cellulase complexes from species of Trichoderma fungi (an organism widely used all over the world for basic and applied studies of enzymatic degradation of cellulose) are usually deficient in cellobiase. In order to increase the yield of glucose, at the expense of cellobiose accumulating in the reaction mixture, it is recommended to enrich the Trichoderma complex by adding cellobiase isolated from another microbial source. On the other hand, cellulase complexes from Aspergillus fungi often have a relatively high content of cellobiase but lack cellobiohydrolase and exoglucohydrolase, both of which play an important role in accelerating cellulose degradation. The composition of some cellulase preparations is shown overleaf.

1. Cultural Liquids (Manufactured in the Soviet Union)

	Activities of Individual Components (I.U./ml)			
	Endoglucanase	Exoglucosidase	Cellobiohydrolase	Cellobiase
Trichoderma reesei 13/10	8.6	2.1	4.8	0.026
T. viride 44	4.8	1.2	1.4	0.046
Geotrichum candidum 3C	0.74	0.14	0.78	1.3
T. longibrachiatum	1.8	0.38	1.3	0.031
Aspergillus terreus 17p	4.2	0.16	0	0.37

Source: Unpublished data provided by the author.

2. Crude Technical Preparations (Manufactured in the Soviet Union)

	Activities of Individual Components (I.U./g)		
Brand Names (source)	Endoglucanase	Exoglucohydrolase	Cellobiase
Celloviridine G3X (T. viride)	57	12	0.5
Cellobronine G3X (T. longibrachiatum)	26	10	5
Cellolignorine PX (T. lignorum)	18	0.8	6
Cellolignorine P10X (T. lignorum)	36	4	12
Cellocandine G3X (G. candidum)	74	7	10
Cellocandine G10X (G. candidum)	130	15	45
Cellokoningine P10X (T. koningii)	120	35	13
Pectofoetidine G3X (Asp. foetidus)	19	<1	50
Pectofoetidine P10X (Asp. foetidus)	50	<1	120

Source: Ref. (3)

3. Purified Preparations

	Activities of Individual Components (I.U./g)		
	Endoglucanase	Exoglucohydrolase	Cellobiase
Trichoderma reesei (NOVO)	3000	350	20
T. longibrachiatum	5500	175	70
Geotrichum candidum	5700	250	90
G. candidum	4000	800	1000
Aspergillus foetidus	190	< 3	3900
Asp. terreus	1160	120	130

Source: Ref. (3) and unpublished data provided by the author.

B. Activity

73. The main technical use of cellulases lies in the total conversion of cellulosic fraction from various cellulose-containing material into glucose. Therefore, it is in the interest of the industrial biochemist to measure the total cellulolytic activity, ie. the activity of the cellulase complex producing glucose from cellulose. Determinations of the total activity, however, are complicated by several factors, related to the nature of both the enzymes and the substrates:

- The enzymes of cellulase complexes often act synergistically, so the activity measured is greatly influenced by the proportion in which various enzymes are present;

- The substrates used, ie. various forms of soluble or insoluble cellulosics, are difficult to standardize macromolecules.

74. The most widely used substrates for determining total cellulolytic activity include filter paper, microcrystalline cellulose, cotton fibres and soluble carboxymethyl cellulose. Each method usually involves measuring the reducing sugars formed during the course of the enzymatic hydrolysis of the substrate. The method of using filter paper hydrolysis, the so-called Mandels-Weber method, has been generally accepted for this purpose. The standard filter paper activity is measured in the filter paper units (FPU), or international units (IU),

which correspond to the amount of cellulases liberating one micromole of reducing sugars measured as glucose per one minute under assay conditions.

75. For the last 20 years Trichoderma viride (= Trichoderma reesei) has been known to be the best source of complete cellulase, that is, cellulase which contains all the necessary components for the hydrolysis of crystalline cellulose. By using this microorganism and by applying biosynthesis optimization and mutation techniques, researchers at the U.S. Army Natick Research and Development Command, Massachusetts, and at Rutgers University, New Jersey have increased enzyme productivities and titers (in terms of total cellulase activity). The highest titer obtained to date, 14.8 I.U./ml, resulted from the fermentation of compression-milled cotton with the NG14 (Rutgers) mutant. Maximum productivity of 167 I.U./l/hr has been recorded through the use of new mutants at Natick.

76. Regarding cellobiase activity in a culture broth, in a prepilot plant fermentation on a chemical pulp hydrolysis syrup, 16.7 I.U./ml of cellobiase from Aspergillus phoenicis (QM 329) was produced at Natick with a maximum productivity of 135 I.U./l/hr over 119 hours.

77. To make these figures more meaningful, note that to produce 11% sugar syrup in 24 hours from compression milled newspaper, the charge to the hydrolysis vessel would be 25% of cellulose with the Trichoderma enzyme to substrate ratio of 10 I.U./gm. In this case the level of reducing sugars is high enough to permit direct practical fermentation to ethanol without an additional sugar concentration step. Then, it was shown that the production of glucose in the enzymatic hydrolysis of ball milled newspaper increases by 25-35% as cellobiase from Asp. phoenicis was added to the cellulase (T. reesei) broth up to 3 I.U./ml.

C. Other Important Properties - Adsorption

78. The properties of cellulolytic enzymes from different organisms may vary to a certain degree in reference to heat stability, the dependence of the activity and the stability on the pH, sensitivity to inhibition and activation by the reaction products, capacity for being adsorbed on the surface of an insoluble substrate, the capacity for transglycosylation reactions (actually side reactions leading to the formation of unwanted by-products), substrate specificity etc. As yet, the relationships between cellulolytic enzyme sources, the nature and scale of variations and reaction conditions are far from being understood. Pertinent studies are still in their infancy. Adsorption of cellulases on the substrate surface will here be briefly considered as one of the most important

properties of cellulases, since it determines the prerequisite step for the enzymatic hydrolysis of insoluble cellulose. As recently realized, the capacity of cellulases for being adsorbed could be extremely important for the biotechnology of the enzymatic hydrolysis of cellulose.

79. It has recently been shown at Moscow State University and the Institute of Biochemistry, Moscow, that similar cellulase enzymes from different microorganisms varied dramatically in their capacity to be adsorbed on cellulose - the difference ranging between hundred and a thousand fold. To achieve the surface concentration of the best cellulases (in terms of adsorption) it is necessary to use hundreds or thousands times larger quantities of "poor" enzymes. This is hardly feasible. Consequently, the higher the adsorption ability of the enzymes, the greater its surface concentration, and the number of enzymes directly participating in the hydrolysis of cellulose, as well as the yield of the end-product.

80. Deeper investigation into the problem showed that most endoglucanases consist of a number (at least two) of isoenzymes whose ability to adsorb on cellulose differs substantially. Two isoenzymes from the same sources may differ a hundred-fold in binding with cellulose. On the other hand, tightly binding endoglucanases of various origin differ in binding by an order of magnitude. Thus, tightly binding endoglucanase from T. reesei and poorly binding endoglucanase from Asp. niger differ in their adsorption constants nearly 1000 times. Clearly, a biotechnologist should keep these factors in mind. For example, in a batch reactor he can use more or less poorly bound cellulases, but not so for a column reactor, where poorly bound cellulases will immediately be eluted from the column by water or buffer.

81. It was shown that the ability of a cellulase to solubilize cellulose is directly correlated with its ability to be adsorbed on it. At the same time, the adsorption itself is identical on both amorphous and crystalline cellulose and is related to the surface of the insoluble substrate. The stronger the enzymes are bonded on crystalline cellulose, the higher the rate of reaction and the greater the yield of glucose, irrespective of the duration of the reaction. Moreover, when cellulases are weakly adsorbed the degree of hydrolysis of cellulose does not exceed 8 - 9% and corresponds approximately to the share of amorphous cellulose in the substrate. Conversely, when the adsorption of the cellulases is tight an almost total hydrolysis of crystalline cellulose takes place. This is illustrated overleaf:

1. The Effect of Adsorption on Endoglucanases from Different Sources on Their Reactivity Toward Microcrystalline Cellulose

Source	Adsorption Constant (1/g)	Conversion of the Substrate, %
T. reesei	0.15	97
T. longibrachiatum	0.12	92
G. candidum	0.093	88
Asp. terreus	0.050	70
Rapidase	0.025	27
Asp. niger	0.020	8.5
Asp. foetidus	0.015	8.0

Source: Ref. (12)

82. The most striking discovery in this respect was the principal difference in the behaviour of amorphous and crystalline cellulose in the hydrolytic reaction. In the case of amorphous cellulose, the degree of its conversion can be increased up to 100% simply by an increase in the enzyme activity or concentration in the reaction mixture. For example, even in the case of cellulase from Asp. foetidus complete hydrolysis of amorphous cellulose was observed, when the enzyme concentration in the solution was sufficiently high. But this was not the case with crystalline cellulose, where the substrate was highly resistant to weakly adsorbed cellulase.

83. Thus, if a cellulase binds weakly with a cellulose the crystalline substrate is virtually resistant to hydrolysis, irrespective of the amount of enzyme used for the reaction. On the other hand, if a cellulase binds tightly the reactivity of the crystalline cellulose increases so much that it can reach 30% of the reactivity of the amorphous cellulose. This property, that is tight adsorption, when coupled with the high catalytic activity of enzymes toward soluble cellulosics, such as CM-cellulose, is crucial for the effective degradation of cellulose. This data indicates that an essential test for cellulases, as well as measuring their enzymatic activity, should be recording its adsorption ability on cellulose.

D. Enzyme Sources

1. Fungi

84. Although many fungi can degrade cellulose, the products of growth are usually only microbial cells and metabolic products such as carbon dioxide and methane. Besides that, numerous fungi degrade soluble cellulose derivatives such as carboxymethyl cellulose, but only comparatively few of them can produce high levels of extracellular cellulases capable of extensively degrading insoluble cellulose to soluble sugars in vitro. These fungi include Trichoderma reesei (= Trichoderma viride), T. koningii, T. lignorum, T. longibrachiatum, Phanerochaete chrysosporium (= Sporotrichum pulverulentum, = Chrysosporum lignorum), Geotrichum candidum, Penicillium funiculosum, P. iriensis, Eupenicillium javanicum, Schizophyllum commune, Polyporus adustus, Fusarium solani, F. lini, Sclerotium rolfsii, Aspergillus wentii, Asp. terreus, Asp. niger, Asp. foetidus. Thermophilic microorganisms are viewed as a source of thermostable cellulases; however, cellulases from thermophiles may not necessarily be more heat-stable than cellulases from mesophiles.

85. Until recently, most of the applied works in the area of the enzymatic conversion of cellulose to glucose utilized strain Trichoderma viride QM 9414 as a source of cellulase. The development of hyper producing and catabolite repression resistant strains T. reesei Rut C-30 and Rut-P37 as well as strains T. reesei VTT-D-80132 and -80133 (VTT Biotechnical Laboratory, Finland), has let to a reevaluation of these processes.

(a) Trichoderma reesei: Relevant Facts

Positive	Negative
- Mutants are effective producers of a variety of enzymes, including those necessary for the hydrolysis of cellulose and xylan.	- The specific activity of cellulases is low.
- The amount of extracellular enzyme protein excreted is exceptionally high.	- End products' inhibition of cellulase is significant.
- Cellulose is not necessary for the production of cellulase.	- Cellulase is not very thermostable compared with the enzymes produced by some thermophilic microorganisms.
	- Catabolite repression is a limitation in the production of cellulases.

- Cellulase is already being produced on an industrial scale in many countries (including the United States, the Soviet Union, Japan and Denmark) and is commercially available.
- Lower cellobiase activities are found than in most other cellulolytic strains.

Source: Adapted from Ref. (2), p. 371.

2. Bacteria

86. The mechanism of bacterial cellulose degradation is possibly similar to that in fungi. The endoglucanases of bacteria are found to be either exclusively cell bound, or extracellular, or both cell bound and extracellular. Bacterial cellobiases, however, are always found to be cell bound.

87. The genus Cellulomonas is among the best characterized cellulolytic bacteria Others are Bacteroides, Clostridium, Pseudomonas, Ruminococcus, Sporocytophaga, Streptomyces, and Thermomonospora. A cellulolytic enzyme preparation from Cellulomonas sp. is quite resistant to end-product inhibition by glucose, cellobiose, xylose and ethanol.

88. An attractive alternative to the production of ethanol is a process in which cellulase production, cellulose hydrolysis and ethanol fermentation are carried out simultaneously in a single stage. When Clostridium thermocellum (a thermophilic and an obligate anaerobic bacterium) is used for this purpose either alone or in coculture with C. thermohydrosulfuricum, C. thermocellum is able to degrade pure cellulose (Solka floc SW-100) directly into ethanol with acetic acid, hydrogen, and carbon dioxide formed as by-products of this fermentation. The yield of ethanol was about 0.3 grams per gram of consumed cellulose. While the continuous system of fermentation as well as the batch method has been shown to be feasible, neither may be economical at the moment because C. thermocellum grows so slowly.

3. Marine Organisms

89. Many marine and fresh-water organisms contain, in their digestive tract, cellulolytic enzymes that degrade cellulose in cell walls of aqueous plants. Cellulolytic enzymes have been found in various types of Coelenterata, Vermes, Arthropoda, Mollusca and Echinodermata. The highest content of cellulases was found in crustacea, gastropoda, bivalvia, and in the giant octopus. The very high activities of cellulases in some marine organisms make them attractive for the commercial manufacture of the enzyme, at least for bench-scale work. This might be economically significant, especially when wastes after processing edible marine organisms like crabs, lobsters, octopuses, squids, snails and oysters are utilized. As shown below, the amount of cellulases per organism is sometimes comparable with that per ml of cultural fluid of a hyper-producing cellulolytic fungus (see paragraph 72.).

(a) Endoglucanase Activity in Tissue Extracts of Some Marine Invertebrates

Phylum	Class	Species	Tissue	I.U./Species
Arthropoda	Crustacea	Hapalogaster dentata [a]	Hepatopancreas	8.3 ± 1
Mollusca	Amphineura	Acanthopleura granulata [b] (chiton)	Digestive tract	0.66 ± 0.04
	Gastropoda	Littorina sp. [a]	Hepatopancreas	1.3 ± 0.1
		Livona pica L. [b] (shails)	Digestive tract	8.2 ± 0.5
	Pelecypoda	Modiolus difficilis [a]	Hepatopancreas	1.5 ± 0.1
		Spondylus americanus [b]	Hepatopancreas	2.4 ± 0.1
		Scapharca broughtoni [a]	Hepatopancreas	1.2 ± 0.1
		Chlamys farreri nipponensis [a]	Hepatopancreas	1.2 ± 0.1
		Patinopecten yessoensis [a]	Crystalline style	0.8 ± 0.1
	Cephalopoda	Octopus vulgaris [a]	Liver (1014 g by weight)	165

[a] The Sea of Japan

[b] Carribbean Sea

Source: Ref. (14) and unpublished data.

4. Ruminants

90. To determine the number and types of cellulolytic organisms present in a complex ecosystem such as the rumen, is a difficult problem. However, there are many bacteria which can ferment cellulose to produce a variety of products, such as ethanol, lactate, succinate, or propionate, apart from the methanogenic precursors acetate, formate, hydrogen, and carbon dioxide. Co-cultures of such organisms with hydrogen - but not acetate-utilizing methanogens-produce methane via hydrogen, accompanied by an increase in acetate production.

91. Until recently the major cellulolytic microbes identified in the rumen were anaerobic bacteria, and they have long been accepted as the main agents of cellulose digestion. However, it was recently discovered that the fermentation of cellulose by the rumen anaerobic fungi also resulted in the formation of ethanol, lactate, acetate, formate, CO_2 and H_2. In the co-culture the major products were acetate, carbon dioxide and methane.

92. Ruminococcus albus is one of the most important cellulolytic bacteria found in the rumen. It is found with the cellulolytic bacteria Ruminococcus flavefaciens and Bacteroides succinogens in proportions that vary according to diet. With good quality diets ruminococci predominate; it is assumed that B. succinogens proliferate with fodders which are difficult to digest. R. flavefaciens and B. succinogenes can ferment the most highly ordered substrates such as cotton fibre; most strains of R. albus, in contrast, can only utilize substrates in which the cellulose is present in a more disordered form.

93. Of the cellulolytic bacteria found in the rumen, only R. albus can be cultured in synthetic media without rumen fluid to yield a reasonably large amount of extracellular cellulase. Reportedly, cell-free cellulases from R. albus can degrade the soluble derivatives of cellulose, or cellulose that has been partially disordered by physical or chemical treatment. However, ordered cellulose is not hydrolyzed to any significant extent. These enzymes have not yet been examined in relation to their behaviour as potential industrial catalysts.

5. Plants

94. There are only a few research projects focusing on cellulases of plant origins. It seems that they contain only endoglucanases; therefore, the ability of plant cellulases to produce glucose from cellulose is negligible

E. Process

1. Glucose Production

95. Cellulase complexes from various sources differ substantially in their composition in relation to the individual cellulolytic enzyme components (see paragraphs 71 and 72). Consequently, the dynamics and yields of glucose formation during the course of the enzymatic hydrolysis of cellulose vary for different cellulase preparations. If a complex is deficient in cellobiase, for example, like most Trichoderma reesei preparations, resulting cellobiose accumulates in the reaction mixture at the expense of the end-product of the hydrolysis, i.e., glucose. For some T. reesei preparations it was reported that, after a reasonable time for the enzymatic hydrolysis, roughly 80% of the soluble reaction products consisted of cellobiose. In that case, to increase glucose yield and decrease cellobiose inhibition of cellulolytic enzymes, it is recommended that the cellulase broth be supplemented with cellobiase from other sources. It is possible this way to increase substantially the yield of glucose from the hydrolysis of cellulose.

96. By using T. reesei cellulase with filter paper activity of 2 - 5 I.U./ml with a pretreated substrate with concentrations of 5 - 25% in weight, a total sugar concentration of between 4 - 10% can be obtained in 24 hours. In this case, the glucose production level can be high enough to permit direct practical fermentation of ethanol without an additional sugar concentration step.

2. Product Inhibition

97. It is difficult, and at present impractical, to raise the total sugar concentration in the Trichoderma hydrolysates above 6 - 8% because the cellulases from this source are inhibited by the reactor products, ie. glucose and cellobiose. Thus, the rate of hydrolysis by T. longibrachiatum cellulase is halved in the presence of 1.1% of glucose or 0.8% of cellobiose, when the amount of cellulose in the reaction mixture is small. With an increase of the substrate concentration the critical level of products, after which the inhibition of the process is substantial, also increases although it hardly exceeds 6 - 10%. This, in turn, leads to a lower productivity of the enzymatic hydrolysis of cellulose (in terms of g/l/hr) at the expense of higher sugar concentration in the reaction mixture. For example, in the hydrolysis of alkali pretreated cotton stalks by T. longibrachiatum cellulase, productivity

of the continuous type reactor was equal to 5 g/l/hr for the steady state level of glucose of 1.8%, but dropped to 2.5 g/l/hr after the flow through the reactor was slowed down to increase glucose concentration at the outlet to 5.5%. A further increase of glucose content in the syrups produced leads to an additional drop in the reactor productivity. Clearly, more research is needed to find a strain producing cellulases with a decreased susceptibility of being inhibited by the soluble products of cellulose hydrolysis. Otherwise it is necessary to compare the cost of glucose production with that of the additional sugar concentration step.

3. Enzyme Recycle

98. During the hydrolysis process, an enzyme can be lost in three ways. Part of the enzyme can be strongly adsorbed on unhydrolyzed cellulose; some of it may remain in the solution containing glucose; and some becomes inactivated during the course of the hydrolysis (as a result of the heat or of the shearing effects produced when an agitating reactor is used).

99. Methods of enzyme desorption, such as those using phosphate ion gradient on cellulose by adjusting the pH to neutrality, may permit greater enzyme recovery. Fresh solids can be brought into contact with the hydrolysate to adsorb some of the enzyme remaining in the solution. Workers at the Natick Laboratories have found that this method of enzyme recovery may not be economical. However, other researchers believe that for each particular substrate methods must be found in order to achieve a satisfactory balance of activities in the blend of fresh and recycled cellulase.

100. Another approach to enzyme recycling is the use of adsorbed cellulase in the continuous conversion of cellulose into glucose in a column reactor. This technology is now scaling-up in the Soviet Union. As cellulose is digested, the released enzyme is readsorbed on excess or newly added cellulose with retention of activity. Adsorption results in a marked economy of enzyme utilization in the continuous process. The substrate can be retained in the column reactor until a high-percent conversion (96% in the case of pretreated cotton stalks hydrolysis, or nearly 100% for paper-making wastes) has been achieved. The sugars are recovered in a clear aqueous solution free of enzyme, cellulose, and any insoluble impurities. The adsorbed enzyme is sufficient to digest the cellulose for a relatively long time; requiring replenishment once every 1 - 2 months in the form of a commercially available culture fluid.

4. Economic Assessment: An Example

101. Economic evaluations of a number of specific scaling-up technologies for producing sugars and ethanol from cellulose are provided in the next section of this paper. An example, the economic assessment recently performed at the University of California, Berkeley, is here considered.

102. The cost analysis is based on a plant with a manufacturing capacity of 10×10^5 gallons/year of 95% fuel grade ethanol. As substrate, 1376 tons of cellulose waste (corn stover) containing 58% glucose equivalent is supplied each day to the plant. Using 7.0 I.U./ml of cellulase, 40% of the substrate is hydrolyzed to fermentable sugars (12 - 15%) which, in turn, are converted by yeasts to ethanol in 46% yields. Continuous countercurrent recovery of the remaining enzyme is accomplished by adsorption on fresh pre-treated substrate. Following filtration, spent solids from the hydrolyser are fed into the furnace of a steam power plant to provide steam and electricity for the process.

103. As summarized below, the economic analysis based on this process suggests a manufacture cost of 10 cents per pound for sugar if the corn stover is free.

(a) Sugar Production Cost (1982)

	Milling	Acid Pre-Treatment	Hydrolysis	Enzyme Recovery	Enzyme Make-Up	Total
Fixed capital x $ 1,000	3,400	5,100	8,700	1,900	10,300	29,400
Annual capital x $ 1,000	810	1,200	1,800	470	2,500	6,780
Annual labour x $ 1,000	96	191	191	96	191	768
Annual utilities x $ 1,000	110	450	660	60	810	2,090
Annual material x $ 1,000	-	1,240	50	-	3,100	4,390
Annual manufacture x $ 1,000	1,000	3,100	2,700	600	6,600	14,000
Glucose, Cents per pound	0.72	2.21	1.91	0.44	4.69	10.00

Source: Adapted from Ref. (17)

104. Assuming the cost of glucose to be 10.0 cents per pound, the processing cost and fixed capital distribution for 95% ethanol production are as follows:

(b) Ethanol Productivity Cost (1982)

	Cents Per Gallon	% Total
Sugar concentration	5.2	2.9
Fermentation	7.6	4.2
Distillation	3.0	1.7
Medium chemicals	21.4	12.0
Glucose	135.9	75.7
Methane generation	6.3	3.5
	179.4	100.0

Source: Ref: (17)

105. If the corn stover is free, the cost of alcohol per gallon is equal to $ 1.80. The predominant portion (76%) of the final ethanol cost is due to the glucose cost. If the price of corn stover is taken into consideration, the minimum glucose production costs shift to lower substrate concentration in the reaction system. This is owing to higher yields of sugars under these conditions as a result of product inhibition.

106. Provided below is a comparison between the lowest manufacturing cost for sugar obtained using cellulase from the strain T. reesei QM 9414 and that obtained from its new descendent mutant T. reesei Rut C-30. Processing with Rut C-30 is a cost saving of 30 - 40% over that obtained with QM 9414.

(c) Cost of Sugar Production at Various Corn Stover Costs (1982)

Corn Stover Cost ($/Ton)	Sugar Cost (Cents Per Pound)	
	QM 9414	C-30
0	14.9	10.5
25	22.9	14.6
50	30.9	18.7

Source: Adapted from Ref. (17)

107. To produce one gallon of ethanol by fermentation, 12.88 pounds of sugar are required. To synthesize one gallon of ethanol from ethylene, four pounds of ethylene are needed. Based on material costs, the price of fermentable sugars must be reduced to approximately one third the price of ethylene which is 10 to 20 cents per pound. In other words, the cost of glucose according to the above estimations has to be reduced by 3 - 5 times if the process is to be economically viable.

108. There is another approach to the problem. Without the concurrent use of pentoses for ethanol production, it is unlikely ethanol production from biomass would be economically feasible, at least not from substrates such as hardwoods and agricultural wastes rich in pentosanes, such as corn stover. Therefore, organisms that can ferment pentoses to ethanol must be found in nature or be developed via the new techniques offered by genetic engineering.

III. SCALE-UP TECHNOLOGIES: CURRENT STATE AND ECONOMICS

109. As yet, no country has an industrial process for the enzymatic conversion of cellulose into sugars or alcohol. Several countries have, however, designed and built pilot or pre-pilot scale solid waste treatment plants. The advantages and disadvantages of the major specific technologies being developed in this area can only be evaluated by comparing them with each other. Outlines for proposed commercial processes and comments on these technologies are as follows:

A. The United States of America

1. Gulf Oil Chemicals Company

110. Gulf Oil Chemicals' cellulose alcohol technology appears to be the most advanced from both the technical and economic viewpoints. It is based on extensive research begun in the early 1970s at Gulf's Merriam Laboratories, and on the 1 t/d pilot plant operation in Jayhawk Works near Pittsburg, Kansas. The process has four major stages: (1) the pretreatment of feedstocks, (2) the in-house production of required yeasts and enzymes, (3) the simultaneous saccharification of feedstocks and fermentation of produced sugars into ethanol, and (4) the distillation of the alcohol. An evaluation of the Gulf technology by Raphael Katzen Association has shown the process to be technically feasible. Furthermore, according to available information it appears that the economic potential of other US enzymatic technologies is not as promising as the Gulf approach.

111. Gulf's proposed commercial process is based on processing 2,000 t/d (dry basis) of cellulosic waste material, containing approximately 57% cellulose. A typical mixed feedstock ($ 15.75/t avg.) composed of 2/3 municipal solid wastes (MSW, the air-classified fraction) and 1/3 pulp mill waste (PMW, primary sludge and digester rejects), is used as the basis for cost estimates. One t/day of the mixed feedstock yields approximately 300 l/day of 95% alcohol. According to available data, the 2,000 t/d cellulosic wastes will yield 150,000 gallons per day of industrial (190° proof) ethanol. The study assumes that the processing facility is located across the fence from a municipal solid waste treatment plant. The MSW is air-conveyed to the cellulose alcohol facility. Pulp mill wastes are transported to the facility by either road or rail.

112. In the raw material preparation section, the feedstock is split between two separate pretreatment steps. Approximately 15% of the MSW is mechanically pretreated and sterilized prior to use as feedstock in continuous enzyme production. The remaining MSW is mechanically pretreated and mixed with incoming PMW, after which the mixture is pasteurized. Afterwards, the mixture becomes the feedstock for simultaneous saccharification and fermentation (SSF).

113. Enzyme production is a continuous process of T. reesei cultivation with a 48-hour total retention time. This process is much faster than conventional long-term batch processes (one to two weeks). The enzymes need not be extracted from the enzyme broth, but can be used directly in the next stage.

114. This next stage is simultaneous saccharification and fermentation, which also has reduced time requirements compared to other processes (most have separate steps for saccharification, which takes off 2 - 6 days, and fermentation, which may take a further two days). The SSF process is carried out continuously in a series of fermenter trains with a total retention time of 24 hours. The multitrain fermenter concept permits the operator to shut down and sterilize one train while maintaining operations in the remaining trains.

115. The beer slurry of 3.5% ethanol content from the SSF stage is neutralized prior to distillation. The alcohol recovery section is designed for maximum heat recovery and heat reuse. The stillage from the alcohol recovery section is evaporated to produce a syrup animal feed by-product with a 60% solids

content. The by-product animal feed production amounts to 534 tons per day and is composed of protein, carbohydrates, inorganics and trace vitamins.

116. Also inherent in the process is the separation of insoluble solids, the organic content of which - primarily lignin and unconverted cellulose - serves as basic fuel for the plant, essentially providing all the thermal energy and most of the motive (turbine drive) energy. By providing most of its own fuel the Gulf process minimizes external fuel requirements.

117. It is noteworthy that the Gulf process does not utilize any acids or solvents, thereby minimizing corrosion and eliminating solvents' recovery problems. However, the investment base and related operating charges are substantial (see below). A 50 t/d demonstration plant, now in the planning stage, is to be built in Pine Bluff, Arkansas, and may in time indicate ways and means of reducing these costs.

(a) 2,000 Tons Per Day Commercial Plant Investment (1981 Costs) - 50 Million US Gallons Per Year 190° Proof Ethanol

	$ Million
Receiving and storage of raw materials	8.95
Preparation of raw materials	11.65
Enzyme production	8.34
Simultaneous saccharification and fermentation	16.80
Alcohol recovery	10.42
Waste liquor (feed molasses) and condensate treatment	13.98
General facilities and off-sites (including dryer, boiler, power generation, cooling tower, water treatment, etc; offices and laboratories, maintenance and stores, alcohol storage and shipping, fire protection, etc.)	27.46
Total Installation + 15% Contingency	97.60
	14.60
TOTAL INVESTMENT	$ 112.20

Source: Ref. (18)

118. As indicated below, the production cost on a 100% investor equity capital basis is $ 0.70 per gallon (18.5 cents per litre) of alcohol. After credit is taken for the animal feed by-product and allowances made for investment tax credit and a 15% after-tax return-on-investment for a 10-year plant life, the projected selling price during the first year of production, 1983, is expected to be $ 1.44 per gallon (38 cents per litre).

(b) Operating Cost and Selling Price Estimates (1983) - 50 Million US Gallons Per Year Production - 10-Year Amortization with Feed By-Product 100% Company Financing

	Annual ($ Million)	$/Gallon Ethanol
Fixed charges	17.97	0.359
Raw materials, chemicals	20.08	0.402
Utilities	10.58	0.212
Labour	2.50	0.100
Total production cost	51.13	1.073
Total operating cost (including by-produce credit)	35.11	0.702
Total income and selling price	71.80	1.436 (38 cents per litre)

Source: Adapted from Ref. (18)

119. The estimated cost of one gallon of ethanol will be almost twice as much if a smaller plant producing 10 million gallons per year alcohol (400 t/d cellulosic wastes) is constructed.

120. Provided below are figures which allow an analyst to compare the cost of producing one gallon of alcohol in a 50-million-gallons-per-year plant via three methods: (1) through SSF of cellulose, (2) through corn fermentation or (3) through synthesis. Each method allows for a 15% after-tax return on investment. Clearly, if all the technical and economic assumptions can be proved valid, the cellulose alcohol process will be competitive in the alcohol market.

(c) Operating Cost and Selling Prices (1983) - 50 Millions US Gallons Per Year 190° Proof Ethanol

	Cellulose	Synthetic	Corn
		($ Million)	
Investment (including working capital)	122.20	57.80	75.80
		($/Gallon)	
Fixed charges	0.359	0.165	0.203
Raw materials:			
Cellulosic wastes, $ 15.75 per ton;			
Ethylene, 18 cents per pound;			
Corn, $ 3.0 per bu	0.208	0.750	1.200
Others	0.193	0.024	0.001
Utilities	0.212	0.238	0.278
Labour	0.100	0.018	0.046
Total production cost	1.072	1.195	1.728
Total operating cost (including by-product credit)	0.702	1.292	1.428
Net profit	0.367	0.174	0.228
Alcohol selling price	1.436	1.640	1.876

Source: Adapted from Ref. (18)

121. In August 1979 the cellulose to ethanol technology developed at Gulf Oil as a proprietary package was donated to the University of Arkansas Foundation. The University then established the Biomass Research Centre whose objectives include continued research and development in biomass utilization and scale-up to a demonstration level. Funding has come from private sources and the US Department of Energy (DOE). Through the efforts of the Biomass Research Centre and representatives from the Cellulose Alcohol Development Company (CADCO), financing has been arranged for a 50 t/d plant to be built in Pine Bluff, Arkansas. Feedstock for the facility will be 37.5 t/d of air-classified MSW plus 12.5 t/d of primary clarifier sludge from a local paper pulp mill. There are also plans to use rice hulls, cotton gin trash, bagasse and straws as feedstock. The plant will produce 5 million litres of ethanol per year. The cost of the plant is expected to be $ 26,000,000. It is assumed that the plant

will operate for a minimum of 2.5 years prior to commercial scale-up. In 1981 the DOE planned to partially finance the construction of the plant by means of a grant worth $ 10.5 million but the action has been postponed.

122. United Biofuels Industries, Inc. (Richmond, Virginia) recently announced plans to commercialize the Gulf-University of Arkansas process by building a 50 million gallons per year plant that will use 2,000 t/d of waste wood pulp and Trichoderma reesei enzymes. Foster-Wheeler Corporation is the overall project manager with Raphael Katzen Associates as designer. The plant will have four individually-built modules, each with a 12.5 million gallons per year capacity. Lignin and heavy combustibles will be burned to generate steam for a condensing steam turbine able to produce 40,000 kW electricity. The cost of the plant has been estimated at $ 130 million ($ 160 million according to another source).

2. United States Army - Natick Laboratories

123. A primary goal of the enzymatic hydrolysis programme at Natick is, in part, the development of technology for producing low-cost, high-quality cellulase complex enzymes. As a result of this work, Trichoderma reesei mutant enzyme productivity has been raised from 8 filter paper international units/l/hr to 167 I.U./l/hr. Additionally, more than 100 different cellulosic materials from sources all over the world and covering the wide spectrum of native and processed cellulose and lignocellulose have been evaluated with respect to enzymatic attack. Out of the many different types of pretreatments, including milling (attritor, ball, colloid, hammer two roll), hydropulping, disc refining, acid, solvents, chemical and steam, two have been found as the most promising; steam and compression (two roll) milling. In prepilot plant studies, a 20% slurry of compression (two roll) milled newspaper was hydrolyzed yielding an 8% reducing-sugar syrup in 24 hours. However, both of these methods have been tested only on a laboratory scale with no indications being given as to whether they are practical or economical on a large scale.

124. The research efforts of the Natick group are also directed toward integrating and optimizing ethanol production with other parts of the process. The programme includes the development and characterization of coupled/uncoupled batch and continuous saccharification and fermentation systems with emphasis on product removal and optimization of the physical process parameters including pH, temperature, time, and concentrations of cellulase, cellulosic substrate and yeast (Candida utilis and Saccharomyces cerevisiae). The fermentation produced 4% to 5% (v/v) ethanol solutions with no apparent adverse effects on the fermentation from urban waste or newspaper derived components. The yield of ethanol was about 45% based on the initial glucose concentration in the syrup.

125. A solid waste treatment prepilot plant has been designed and built by the Natick group in conjunction with the New Brunswick Scientific Company, Inc., New Jersey, to convert cellulose wastes to sugars and alcohol. A mutant strain of T. reesei is cultivated in a 400-litre fermentor for the production of cellulase in submerged culture. The cellulase is transferred to a 250-litre enzyme reactor where a substrate is converted to carbohydrates and ethanol. This enzyme hydrolysis facility is highly instrumented to permit extensive analysis and a high degree of control of the physical process parameters. As a result of specific rates and yield factors obtained with the prepilot plant facility the economics of the process has been evaluated.

126. The cost analysis is based on a plant with a manufacturing capacity of 25 million gallons per year of 190° proof fuel grade ethanol. A material balance on the plant shows that 495,000 tons of urban waste containing 375,000 tons of enzyme hydrolyzable cellulosics is supplied each year. Using 5 - 10^{12} I.U. of cellulase, or 1 million m^3 of the cultural fluid with the activity of 5 I.U./ml, 45% of this substrate is hydrolyzed to fermentable sugars (10% syrups) which in turn are converted by yeast to ethanol in 40% yields. The plant operates 24 hours per day, for 330 days per year; an on-stream factor of 0.9. Other figures are as follows:

- Cellulase productivity of 125 I.U./l/hr;
- The enzyme is used for only one hydrolysis period of 24 hours owing to economic considerations;
- Initial substrate solids' charge of 20% is increased to an effective 30% level by the addition of substrate during the first few hours of hydrolysis;
- The ratio of enzyme to urban waste derived substrate is 10 I.U./gm;
- Energy required for ethanol distillation is 25,000 Btu/gallon based on the recent (1979) commercial plant designs for the production of fuel grade ethanol from corn;
- Pretreatment of the substrate is compression (two roll) milling with 0.225 Kwhr/lb of cellulosic material.

127. The economic analysis (see below) indicates that the factory cost of ethanol from urban waste is $ 1.60 per gallon including $ 0.17 per gallon for the purchase of substrate from urban waste. Enzyme production contributes the most (38%) to the factory cost followed by ethanol production (23%), pretreatment (18%), substrate (11%) and hydrolysis (11%).

(a) Operating Cost and Factory Cost Estimates (1983, $ Per Gallon) - 25 Million US Gallons Per Year 190° Proof Alcohol

	Enzyme Production	Pretreatment	Hydrolysis	Ethanol Production	Total
Raw material, chemicals	0.287	0.008	0.017	0.025	0.337
Utilities	0.078	0.183	0.030	0.111	0.402
Labour	0.064	0.038	0.038	0.060	0.200
Plant overheads	0.051	0.031	0.031	0.044	0.157
Fixed charges	0.119	0.033	0.055	0.121	0.328
TOTAL	0.599	0.293	0.171	0.361	1.424
Cellulose substrate					0.173
Factory cost					1.597

Source: Adapted from Ref. (11)

128. This factory cost of ethanol ($ 1.60 per gallon) is higher than in the Gulf cellulose alcohol process ($ 1.07 per gallon), (see paragraphs 118 and 120). If credits are taken for process steam (enzymatic hydrolysis residue from the ethanol facility can be returned to the utility for use as fuel) and cellular biomass ($ 200/t), factory cost could be substantially reduced. It is anticipated that cellular biomass would be used as animal feed and/or fertilizer. By taking both credits, ethanol factory costs are reduced from $ 1.60 per gallon to $ 1.23 per gallon.

129. Total capital investment for the plant is projected to be $ 85,330,000, as shown below:

(b) 1,500 Tons Per Day Commercial Plant Investment, 1983 Costs - 25 Million US Gallons Per Year 190° Proof Ethanol

	1983 ($, Thousands)
(Total plant equipment)	(20,950)
Battery limit investment	53,160
Off-site investment	8,900
General services facilities	6,210
TOTAL FIXED INVESTMENT	68,270
Start-up (8.5% TFI)	5,800
Working capital (16.5% TFI)	11,260
TOTAL CAPITAL INVESTMENT	85,330

Source: Adapted from Ref. (11)

130. Researchers at Natick intend to develop the process for commercial use in two steps. Urban waste is to be used as the primary substrate and fuel grade ethanol is the end-product. First, a one to two tons per day pilot plant facility is expected to be leased for four to six months to develop process refinements and collect the necessary design data for a 50 tons per day pilot plant. The next step is to build a 50 tons per day pilot plant and conduct a two-year programme to establish the engineering baseline for the design and construction of full scale plants likely to follow.

3. Purdue Process

131. The overall process being developed at the Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, Indiana, includes six sub-areas: hydrolysis of hemicellulose; fermentation of hemicellulose hydrolysate, hydrolysis of cellulose; fermentation of cellulose hydrolysate; recovery of alcohol and other products; treatment of waste liquors in methane generation. According to data supplied by the principal investigators, the programme succeeded in raising funding of $ 440,000 during 1979 - 1980. Some pilot plant equipment has been installed.

132. The principal feature of the Purdue process is that it greatly improves the solvent pretreatment of cellulosics to the point where good yields are obtained by acid or enzymatic hydrolysis. The cellulose solvents used for this step are Cadoxen, 70% sulphuric acid (at 100°C) or zinc chloride solution. Researchers believe that some other solvents (like organic bases, such as amines, eg. ethylene diamine) also have potential. For reasons of economics and/or safety, sulphuric acid seems to be the solvent of choice at this stage of development. Corn stover is the preferred substrate.

133. The process starts with hemicellulose hydrolysis by dilute sulphuric acid at 80 - 100°C to yield xylose and other soluble products, mainly pentoses. A technique known as "roasting and leaching" has been developed involving the use of a minimum amount of water so that a sugar concentration of about 15% (w/v) can be achieved. The pentose solution, or part of it, is then converted into butanediol at 90% theoretical yield in concentrations of about 100 grams per litre.

134. In the next step of the process, the cellulose content in the lignocellulosic residue from the pretreatment operation can be hydrolyzed to yield glucose by a number of different techniques including the classical dilute acid process under severe conditions or the new solvent method (paragraph 132), followed by acid or enzymatic hydrolysis. A 50 to 90% yield of glucose can be obtained. The dilute glucose solution is recycled upstream to hemicellulose hydrolysis. For every kilogram of sulphuric acid consumed it is possible to produce 3 - 3.5 kg of fermentable sugars. The ratio between glucose and xylose varies depending upon the raw material but averages about 2:1. The combined glucose and xylose can be fermented to ethanol. The solid residue consisting of residual cellulose and lignin can be dried and burned to generate steam. With a 50 to 70% hydrolysis of available cellulose, enough residue is produced during operations to support the energy requirements of the whole process.

135. Other accomplishments of the Purdue process are the development of better dehydration methods for ethanol with the successful use of cracked corn, corn starch and other materials as dehydrating agents. Further, a new bacterial strain has been found capable of producing ethanol or butanediol or both from pentoses thereby increasing the alcohol yield by nearly 300%. Enough moisture is adsorbed by passing 180 - 190° proof alcohol vapour through very dry ground corn (1 - 2% moisture) to give 199° proof alcohol. Corn has been dried and reused as many as 20 times.

136. This process is now being scaled-up to larger size laboratory operations. According to the developers of the process, the estimates for producing 40 gallons of ethanol per ton of dry residues appear conservative; between 70 and 75 gallons per ton is economically feasible and 100 gallons per ton are possible. The investigators are now doing a plant design based on overall biomass utilization, including cellulose, hemicellulose and lignin.

4. Berkeley Process

137. Studies undertaken at Lawrence Berkeley Laboratory, University of California, based on small-scale laboratory checks of key process steps coupled with engineering assumptions as yet untested, have resulted in several tentative processing schemes. Therefore, the proposals of the Berkeley group, even though developed for a large plant, are conceptual in nature and reported flows, balances and costs must be taken in this context.

138. The Berkeley group has investigated dilute acid pretreatment of corn stover and wheat straw, which removes hemicelluloses and opens up the cellulose for enzyme hydrolysis. Batch hydrolyses were conducted on the acid treated material using cellulase from batch cultures of T. reesei Rut C-30, for substrate concentrations of 5 to 25% in weight. After 48 hours of the hydrolysis a maximum conversion of approximately 52% of the pretreated corn stover was obtained for the 5% substrate case at enzyme activities beyond 1 I.U./ml.

139. An enzyme recycle stage was developed whereby part of the enzyme was recovered from the product stream by adsorbing cellulases countercurrently on the on-coming, pretreated cellulose solids. Hence a continuous hydrolysis stage is coupled with an enzyme recovery stage, thereby reducing the amount of make-up enzyme required. The cellulose solids, after adsorbing as much of the enzyme as possible, are then passed on to the enzymatic hydrolysis stage. As a result of the research work, a multi-stage continuous countercurrent adsorption process for enzyme recovery has been developed. In such a system the cellulose solids remain stationary in each tank and are brought into contact, by means of vigorous stirring, with the enzyme-sugar solution. The wetted solids are then filtered and brought into contact with fresh enzyme sugar solution, while the filtrate is brought into contact with fresh solids. Preliminary data has shown that in an eight-stage system, it would be necessary to supplement the recycled enzyme with 35% of the enzyme added to the initial charge. Approximately 87% of the filter paper activity leaving the first stage was

present in the exit stream from the eighth stage, and thus available for recycling to the first hydrolysis stage. With this kind of process the authors recover around 8% glucose with total reducing sugars making up approximately 16%.

140. According to the author's estimates of the cost of a 10% glucose solution (but not including the cost or the raw material), producing the enzyme costs approximately 60 cents per equivalent US gallon of alcohol, which would be made using a continuous process with cell recycle. The hydrolysis and recycle add a further 60 - 80 cents, thereby amounting to some $ 1.40 per gallon. If one adds raw material costs, the total comes to well over $ 2.50 per gallon.

5. M.I.T. Process

141. The essence of the M.I.T. process is that carefully selected mixed cultures are added directly to coarsely ground cellulosics. Enzymes hydrolyze both the cellulose and the hemicellulose while the organisms convert the resulting sugars into ethanol. The remarkable feature of this approach is that only a small investment is required in preparing feedstocks and the process is not overly dependent on a high efficiency of feedstock utilization. The residue can be burned to supply energy for the factory; steam or electricity are products of roughly equal importance to the ethanol. Other accomplishments are the development of microbial cultures with improved performance as a result of increasing the microorganism's ability to tolerate ethanol; continuous removal of butanol from the acetone/butanol process of bioconversion of biomass by extraction with a water-immiscible solvent during the reaction; and producing acrylic acid (an important intermediate in the manufacture of plastics and resins).

142. For the direct production of ethanol from agricultural cellulosics, mutant strains of the anaerobic, thermophilic bacteria Clostridium thermocellum and Clostridium thermosaccharolyticum (see paragraphs 86 - 88) have been used. Through strain improvements for increased ethanol tolerance and catabolite selectivity, alcohol yields of 85% of the theoretical maximum have been obtained with mixed culture.

6. Lehigh/Pennsylvania/General Electric

143. A team effort of groups at Lehigh University and the University of Pennsylvania in conjunction with the General Electric Company, Hahnemann Medical College and the Biology Energy Corporation has led to the development of a promising process based on solvent pretreatment in which aqueous solutions

of either butanol or ethanol and a catalytic agent remove lignin from the lignocellulosic feedstock, thereby improving substrate susceptibility to hydrolysis. A new thermophilic culture, Thermomonospora, is used for production of the cellulases. In addition, significant credits may be realized by such measures as utilizing lignin by-products in alcohol solutions such as diesel fuels.

144. The research group has paid considerable attention to feedstock costs because they represent a substantial fraction of the total cost of produced alcohol. Accordingly, nurseries in Pennsylvania have developed short-harvest-cycle, high-yield poplars resulting in feedstock costs of under $ 15 per dry ton. It is particularly interesting that the material produced from harvesting two or three year old trees has about 20% of fines which can be easily separated by air classification. This fraction has 24 - 27% protein and an estimated price of $ 150 to $ 200 per ton for animal feed. Two specific process alternatives being investigated by the research team are: combined saccharification-fermentation using cellulases from Thermomonospora and the thermophilic, anaerobic noncellulolytic bacterium C. thermohydrosulfuricum for ethanol production - and extractive fermentation for both ethanol (S. cerevisiae) and butanol (C. acetobutylicum) production

7. E.G. and G. Idaho, Inc./Colorado State University

145. The group intends to design and construct a pilot plant in Idaho Falls, Idaho, for the enzymatic conversion of lignocellulose, primarily from wheat or barley straw, into ethanol. So far, the autohydrolysis process has been optimized for wheat straw, and more work is in progress to optimize the autohydrolysis of hemicellulose and "organosolv" extraction of lignin from pinewood chips and corn stover. A further expectation is to use batch or continuous digester technology from the paper industry for the autohydrolysis step. Other advances include an improved enzyme cycle system, advanced fermentation work, incorporation of vapour recompression distillation techniques, and the conversion of hemicellulose-based pentose sugars into butanol or another product.

8. University of Lowell, Massachusetts

146. The intention is to develop a practical process for producing alcohol fuel from waste paper. On the pilot plant level, the process will combine modern pulp and paper technology with the use of purchased enzymes from Trichoderma reesei. Mechanical reduction is accomplished by combining waste paper or mill effluent solids with either water or recycled enzyme solution in a Jones system

hydropulper. To enhance the enzymatic hydrolysis, the hydropulper breaks down the paper structure into individual fibres or fibre fragments, which are further reduced to 150 mesh size or less. Enzymatic conversion takes place in a 600-gallon, temperature-controlled, agitated tank. The saccharification stage is 90% efficient. Ethanol is recovered after final distillation as a 180° proof (90% alcohol) product.

B. Canada

1. Iotech Process

147. The Iotech Corporation Limited, Ottawa, Ontario, is a Canadian-owned company, founded in 1975. Iotech has underway a multidisciplinary programme to investigate the conversion of cellulosic feedstocks into ethanol, chemicals and feed, and to commercialize the process. According to available data, the work is financed by US$ 1 million per year from private investors and government grants; the DOE has provided $ 397,000.

148. The Iotech process uses steam explosion for the pretreatment of lignocellulosics (see paragraphs 66 - 70), producing as an essential component a high quality lignin suitable for chemical production. The lignin is sterilized powder, and can be converted to resins, plastics, detergents and petrochemicals. A one ton per day pilot plant, designed and built by the Company, uses aspen chips as feedstock and converts 90% of cellulose and 80% of hemicellulose into sugars. The glucose obtained is converted into ethanol at 95% of the theoretical yield. Currently, a yield of 68 gallons of ethanol per ton of wood have been achieved. If a feasible technology for xylose fermentation is developed by Iotech, the yield is likely to approach 90 gallons per ton.

149. A detailed design of a 250 tons per day (7.5 million gallons per year) demonstration plant and a preliminary design for a 1,000 tons per day (30 million gallons per year) commercial plant have been prepared. The 1980 market price of $ 1.70 per gallon (1983, $ 2.08 per gallon) of ethanol was projected as achievable assuming lignin is burned as fuel. A 250 tons per day demonstration plant has been scheduled for construction in the United States by the mid-1980s; a similar one is planned for Canada.

2. Stake Process

150. Stake Technology Limited, Ottawa, Ontario, has become well known following the disclosure of its efficient, low-cost conversion of waste lignocellulosics biomass through continuous "autohydrolysis". The Company's patented process and equipment accepts waste biomass, such as hardwood chips, sugarcane bagasse or straw, without using any chemicals or other additives. Raw materials are fed, on a continuous basis via a plug-forming feeder, into a steam-pressurized cylindrical vessel with steam pressures of 500 pounds or greater containing a helical screw conveyor. Autohydrolized materials are discharged intermittently to atmospheric pressure through an orifice. The product is then subjected to an aqueous extraction, followed by an alkaline extraction, resulting in the recovery of a raw material containing three major components: cellulose, hemicellulose, and lignin. The cellulose fraction is further saccharified by acid or enzymatic hydrolysis to glucose which is fermented to ethanol. Pentose-rich hemicellulose fraction can be converted into furfural, xylitol or undergoes a specific fermentation to ethanol. The lignin fraction has high fuel value or it can be used as a starting material for producing chemicals. Alcohol yields greater than 80% theoretical have been achieved.

151. A joint venture has been formed between Stake and Technip (France's largest engineering and construction firm) to integrate engineering, construction and marketing activities for alcohol plants outside North America. Vulcan Cincinnati has exclusive license for the use of Stake's technology within North America.

3. University of Toronto

152. The objective of the group at the Department of Chemical Engineering, University of Toronto, is to develop and optimize an autohydrolysis extraction process in order to use under-utilized woods and agricultural residues to produce alcohol. Steps in the basic process are: autohydrolysis; hemicellulose extraction; lignin extraction; hydrolysis, clarification of the wood sugar solution; fermentation and distillation. According to the research group, acid hydrolysis is preferred in most cases. Some aspects of the process are now at the pilot stage.

153. The Toronto University programme is concerned specifically with the problem of sugar survival in acid hydrolysis. Partial acid hydrolysis is studied with as low as 20% conversion of glucose. The remainder is recycled. With this process, glucose yields of 70 - 80% are possible. Another method of hydrolysis

under study is enzymic. A partial hydrolysis-recycle process has been investigated, similar to that proposed for acid hydrolysis, to overcome the problem of glucose inhibition. The research group concluded that this recycle reaction will become practical when immobilized enzymes become available.

4. Canertech Inc.

154. The interests of the Canadian crown corporation Canertech Inc. lie in energy conservation and in renewable energy development. Part of the latter interest is its Ethanol-From-Cellulose Programme, which is a special project within the National Energy Programme. Initially, the Programme aimed to complete a 1 - 5 tons-per-day pilot plant by April 1984 and a 50 - 100 tons per day demonstration plant in 1986/1987. The estimated cost of the overall programme is $ 21,000,000 with $ 7,000,000 ear-marked for the pilot plant. It has now become clear that the need for extensive process technology development will delay the pilot plant by about a year.

155. Detailed, comparative assessments of five alternative acid hydrolysis processes have been undertaken with the Programme aim to produce fuel ethanol competitive in price to petrol. As to the economic aspect, the Company found that by using aspen feedstock, making reasonable assumptions about process development and giving no commercial credit to lignin three of the five processes could lead to a competitively-priced fuel in Canada by 1990. Technically speaking, two of the processes, though in early stages of development appear promising. One of these may have an economic edge and is, therefore, likely to be selected for pilot scale development. However, proprietary complexities associated with one of the two processes have already delayed selection, and may eliminate that particular alternative from further consideration. It is expected sufficient work will have been done on two processes by the end of 1983 to indicate which is ultimately suitable for actual pilot plant operation.

C. The Soviet Union

1. Celloglucose Process (A.N. Bach Institute of Biochemistry/Moscow State University/Research Institute of Bioengineering)

156. The National Programme in Biotechnology, started at the end of 1981, calls for the large-scale production of glucose in the form of glucose syrups and crystalline glucose by means of enzymatic hydrolysis of cellulosic wastes. The development of an appropriate industrial level process should be attained by the end of the 1980s. Work along this line is co-ordinated by the Commission

of Biotechnology of Cellulose at the USSR Academy of Sciences and by the National Council on Biotechnology.

157. The Programme aims to complete by mid 1984 two pilot plants capable of producing 100 kilogrammes of crystalline glucose per day and by 1985/1986 two demonstration plants producing 1 - 5 tons per day of crystalline glucose. At the moment a continuous prepilot plant is running at the A.N. Bach Institute of Biochemistry, USSR Academy of Sciences, producing glucose, ethanol, fructose, and fodder yeasts from cellulosic wastes. Some raw materials may be used directly including cotton stalks and sawdust; others require chemical pretreatment, for example, cotton linters and other cotton ginning residues, and under-utilized slurries from paper-making consisting of tiny cellulose particles. Enzymes used by the pre-pilot plant are solution regularly donated by the nearest industrial biochemical unit which produces 600 m^3 per year of cellulase culture in the form of a non-concentrated microbial culture fluid with a filter paper activity of 1 I.U./ml. The original hydrolyzer unit is a countercurrent continuous reactor with an average glucose productivity of 2 - 5 g/l/hour. The productivity of the reactor can be increased, according to bench-scale experimental results, up to 15 g/l/hr which corresponds to approximately 24,000 tons per year of glucose per industrial 200 m^3 reactor. The cost structure shows that the three major components (raw materials with their pretreatment, enzyme production and use, and the purification and crystallization of glucose) give approximately equal input into the cost of the crystalline glucose production.

158. The high-efficiency, continuous process of enzymatic conversion of cellulose into glucose became possible as a result of the detailed investigation into regularities of cellulases' adsorption on cellulose. As a result, the optimal conditions were found for the adsorption of the enzymes on pretreated cellulosics. The adsorption was found to be so tight that it enabled the researchers to choose a column-type reactor for scaling-up the process. Other accomplishments are:

- The development of specific methods of determining activity (in absolute, international units) for all four principal components of the cellulase complexes (see paragraphs 28 - 30, 71 - 72) without their resolution;

- The development of a kinetic theory of action of multi-enzyme cellulase system on cellulose useable to predict the kinetic behaviour of glucose formation from cellulose while taking into account the activity of individual components of the cellulase complex;

- An increase in knowledge about regularities of adsorption of different cellulases (from different microbial and other sources) on cellulose and the quantitative evaluation of the availability of the enzymes for effective hydrolytic action toward cellulosics;

- A clarification of the role of adsorption of cellulolytic enzymes on cellulose in the effectiveness, on the one hand, of the conversion of amorphous cellulose and crystalline, on the other, into glucose;

- The development of a formula which relates the quantitative relationships between the major physico-chemical and structural factors of cellulose and the effectiveness of its conversion into glucose.

159. These approaches have allowed researchers to make a proper selection of the microbial source of cellulases and cellulosic materials for practical conversion of cellulose into glucose. At the moment the USSR programme (utilization of sugar syrups from refuse) is being developed with the aim of providing resulting sugars ("celluglucose") to various branches of the food industry, microbiology, energetics and chemical industry.

D. India

1. The Indian Institute of Technology

160. At this Institute a group is developing an integrated approach for the bioconversion of lignocellulosics into sugars, organic feedstock and liquid fuels. Within the framework of this objective, two pre-treatment processes for lignocellulosics have been developed. The first, a two-step process, involves dilute alkali treatment followed by steam treatment at 120°C in the presence of alkali. The second employs the treatment with butanol as a catalytic solvent (see paragraph 143) for effective delignification. It has been shown that hemicellulose, the solvent and lignin can be recovered to the extent of 95, 96 and 80%, respectively, by using steam distillation and solvent extraction.

161. Furthermore, a process of simultaneous saccharification and fermentation has also been developed. Using cellulase and Pichia etchelsii at 40°C, ethanol yields of up to 32 grams per litre were obtained at 140 grams per litre of bagasse concentration. As a third approach, the direct conversion of cellulose

into ethanol and other chemicals by Clostridium thermocellum bacterium is also being studied. Ethanol yields of 0.20 and 0.25 g/g substrate degraded have been observed using raw and mild alkali pretreated bagasse. A process is being developed to utilize the xylose component in bagasse (about 30%) via enzymatic isomerization to xylose and subsequent conversion of both glucose and xylose into ethanol. The energy requirements for producing 190° proof (95%) from a feed concentration of 4% have been estimated to be nearly half that of the distillation process.

E. Finland

1. Technical Research Centre/Helsinki University of Technology/State Alcohol Monopoly

162. A project was undertaken to develop an industrial process for producing ethanol from wood and other cellulosic materials based on enzymatic hydrolysis. Research includes the development of mutants T. reesei for the production of cellulases, cellobiases and hemicellulases; pretreatment of cellulosic materials; hydrolysis of pretreated cellulosic materials; enzyme recycling; fermentation of glucose to ethanol by yeasts or bacteria (mainly Zymomonas sp.) in a separate process or simultaneously with hydrolysis of cellulose, fermentation of pentose to ehtanol by moulds (Fusarium sp.); and process design. The production of cellulolytic enzymes has been studied up to pilot scale and tested at an industrial fermentation plant. According to the investigators, the economic feasibility of the processes based on enzymatic hydrolysis of cellulosic materials is as yet uncertain.

F. Sweden

163. The well-known work of the Swedish Forest Products Research Laboratory, Stockholm, has been focused on enzyme mechanisms involved in fungal cellulose and lignin degradation. For the fungi Phanerochaete sp. and Trichoderma reesei, the pattern of attack on cellulose has been elucidated in some detail. In addition, a group at the Biomedical Centre, University of Uppsala, has for a long time been concerned with studying cellulases from Trichoderma strains with the general aim of obtaining results that would be of the greatest possible value to applied research. In recent years, amino acid sequence determination of the individual cellulolytic components as well as their X-ray crystallographic studies have been started by the Uppsala group. Currently the Swedish

Forest Products group in co-operation with a major Swedish company is trying to develop a process for ethanol production based on biomass. The project has recently started and is still at an investigative stage.

G. Italy

1. Comitato Nazionale Energia Nucleare, Rome

164. The objective is to develop advanced biological technologies for converting cellulosic materials into ethanol. The cellulosic wastes under study are textile wastes, paper pulp, wheat straw, corn cobs, and olive shoots. The pretreatment is usually made with sodium hydroxide at low temperatures. The saccharification of cellulosics is performed by the mixture of purchased Trichoderma viride and Aspergillus niger cellulases, the latter being immobilized in gelled alginate beads. An improvement of conventional batch fermentation has been achieved by continuous fermentation with immobilized living cells packed in a column reactor; Saccharomyces cerevisiae cells were immobilized using 10% (w/v) suspension in 3% sodium alginate. Ethanol was produced continuously from a medium containing 15% (w/v) glucose with a yield 90% of the theoretical. The work has so far been performed on a bench scale.

H. South Africa

165. The Council for Scientific and Industrial Research in Pretoria co-ordinates a national programme for converting cellulose and hemicellulose from bagasse into liquid fuels and chemicals. Of the three million tons of bagasse (with cellulose contents of 43 - 45%), 1,300,000 tons per year of cellulosic fraction could be recovered. The conversion of this material into ethanol could contribute 10% of the country's liquid fuel needs. The following research organizations in South Africa are involved in the part of the bagasse programme dealing with the bioconversion of cellulosics into alcohol:

- National Food Research Institute, Pretoria: Isolation and improvement of aerobic cellulolytic organisms; optimization of cellulase production on a pilot scale and the development of large-scale enzyme production process technology; a promising technology using T. reesei QM 9414 or MCG 77 is planned for transfer to a pilot scale 150-litre fermenter;

- University of Natal, Pietermaritzburg: Hydrolysis of untreated and pretreated bagasse by using T. reesei or mutant strains of locally isolated microorganisms; fermentation of the resulting glucose and pentoses to ethanol;

- Sugar Milling Research Institute, Durban: Pretreatment of bagasse to enhance enzymatic hydrolysis;

- University of Orange Free State, Bloemfontain: Enzymatic saccharification of acid extracted bagasse; fermentation of glucose and pentos to ethanol;

- University of Fort Hare, Alice: The study of the cellulase complex of T. reesei;

- University of Cape Town: Ethanol fermentation from bagasse hydrolysate.

I. Japan

166. Ethanol production from cellulosic materials has been established in Japan as a national project of the Ministry of International Trade and Industry since 1980. Until 1986 the project will be worked on by 12 companies belonging to the Research Association for Petroleum Alternatives Development paying particular attention to the development of a practical delignifying process, the physical or chemical pretreatment of cellulosics, the isolation of active cellulolytic microorganisms, the economical production of cellulase, and innovative ethanol separating methods as well as the selection of new cellulosic biomass and the cultivation of it in large quantities.

167. Japanese researchers have attempted to devise a very simple procedure for enzymatic saccharification of cellulosic materials with the intention of producing sugar and ethanol at a low cost and with a considerable saving of fuel. They claim that the production of 10 - 20% sugar solutions comparable to extracts of sugar cane and sugar beet is now feasible from cellulosic

resources, such as rice straw, grass, bagasse, sawdust, corrugated paper and newspaper, by saccharification with T. viride cellulase preparation. Cellulases are recycled during the course of hydrolysis by means of ultrafiltration or by the locally developed tannic acid-polyethylene-glycol method. The simultaneous saccharification-fermentation at temperatures of 15 - 30°C in opened tanks in the presence of citric or lactic acids for preventing microbial contamination, ie. the Oriental brewing method, gives 5 - 7% ehtanol solutions after a 7-day incubation. According to the researchers this is now a practical way of using sugar solutions from cellulosics. The experiments have apparently been performed on a laboratory scale.

168. On a relatively larger scale, the chemical delignification (by boiling with a 1% sodium hydroxide solution for 3 hours or by autoclaving at 120°C with a 1% NaOH for one hour) of eight tons of air-dried rice straw usually yields four tons of holocellulose. After enzymatic saccharification of the holocellulose, one tone of Candida utilis yeasts or a corresponding amount of ethanol is obtainable while providing 50% saccharification. On the other hand, the non-hydrolyzable residue provides two tons of a mixture consisting mainly of lignin, which when ploughed into the soil, is useful as a new compost along with two tons of the residual root and stump of rice plant.

169. For the saccharification of delignified Carpinus shavings or delignified sawdust the economic substrate concentration was estimated as being around 9 - 10%. After four days of incubation with 1 - 3% T. viride cellulase solution at 45°C the sugar concentration was 8.6%, and the degree of saccharification 77%. In another series of experiments, 25% shredded tissue paper, delignified rice straw, or delignified bagasse was incubated with 1 - 3% T. viride cellulases at 45°C for four to eight days yielded 13 - 22% sugar solutions (with a 40 - 66% degree of saccharification). These experiments have been performed on a laboratory scale. Whether there are scaling up technologies and whether their economic assessments have been done is not clear from the available sources. It seems, however, that the high enzyme concentrations and the long incubation time results in an uneconomical outcome.

IV. POTENTIAL FOR BIOTECHNOLOGY OF CELLULOSE IN THE DEVELOPING COUNTRIES

170. The shortage of food and fuel has become more and more serious in many developing countries owing to the congestion of population. From this viewpoint the enzymatic saccharification of cellulosic materials, mainly renewable agricultural residues, is a counter-measure against this problem. One positive feature along this line is the extremely large quantity of cellulosic resources being produced each year in subtropical and tropical developing countries.

171. In those developing countries where the technique of microbial technology is not fully established, enzymatic hydrolysis of cellulosic materials and fermentation of the resulting sugars into ethanol should apparently be performed on a relatively small scale using facilities which are readily available at the outset.

172. In some cases, the existing branches of industry typical for developing countries can afford cellulosic material as wastes which are ready for bioconversion and, moreover, which could, when utilized, be beneficial for the industry. For example, in the manufacture of tobacco substantial amounts of waste filter material and cigarette paper are generated. Such waste materials generally find no use in cigarette manufacture, but instead are typically disposed of by burning after separation from tobacco components. On the other hand, sugars, particularly glucose, are employed in a number of tobacco treatment processes, like a carbon source during tobacco fermentations. In addition, sugars may be employed as tobacco casing materials or in the production of tobacco flavourants.

173. Of the numerous cellulosic residues, rice straw is one of the most promising renewable resources in many developing countries, particularly for those in South-East Asia. Cotton and sugar cane residues are also promising materials, because they have been studied extensively and findings written up in a vast number of publications. With the experience accumulated in the literature it is easier to select an appropriate approach to processing the materi l. Then, as a highly promising way for the pretreatment of lignocellulosics, particularly for the developing countries, the biological pretreatment which uses lignin-utilizing microorganisms (paragraphs 60 - 62) should be considered. The potential for this approach is far from being exhausted even in the industrial countries

which have the opportunities of using highly instrumented, capital-intensive equipment for the pretreatment of lignocellulosics. By all means, new and more efficient celluloseless lignin-utilizing microorganisms could be discovered in natural conditions, tropical or elsewhere, thereby increasing the efficiency of the biotechnological processing of lignocellulose in the developing countries.

174. Clearly, commercial cellulase preparations, such as T. reesei cellulases, are too expensive for the developing countries to purchase for the saccharification of their cellulosic resources on a more or less large scale. The cellulases producible by solid culture using wheat bran, rice straw and so on and solid culture methods rather than submerged cultivations, are recommended for fungal cellulase production in the developing countries, at least for the initial period of accommodation with modern biotechnology. Such cultures have been successfully developed in Japan and the Soviet Union, and show good results in terms of both enzymatic activity and costs.

175. It seems that enzymatic saccharification of cellulose followed by a refinement or succeeding conversion of producing glucose into edible sugars, like fructose, or by the fermentation of the sugard obtained into liquid fuels, or the conversion of it into fodder, will gradually become a new rural industry in the developing countries. From a practical standpoint, such an industry should be operated by using simplified apparatus along with manual labour and must provide employment to a large number of rural people. There is great hope that renewable cellulosic resources will be used as industrial raw material for producing sugar, yeast, and liquid fuel in the near future. Actually, education may well prove to be the critical determinant for future developments in the area covered by this paper.

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